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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 April 2003 (24.04.2003)

PCT

(10) International Publication Number
WO 03/033813 A1

(51) International Patent Classification[7]: D06M 16/00, 101/06, 15/03, C12N 9/10, C08B 37/00

(21) International Application Number: PCT/IB02/04567

(22) International Filing Date: 16 October 2002 (16.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

0103446-1	16 October 2001 (16.10.2001)	SE
0103447-9	16 October 2001 (16.10.2001)	SE
0202310-9	23 July 2002 (23.07.2002)	SE

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

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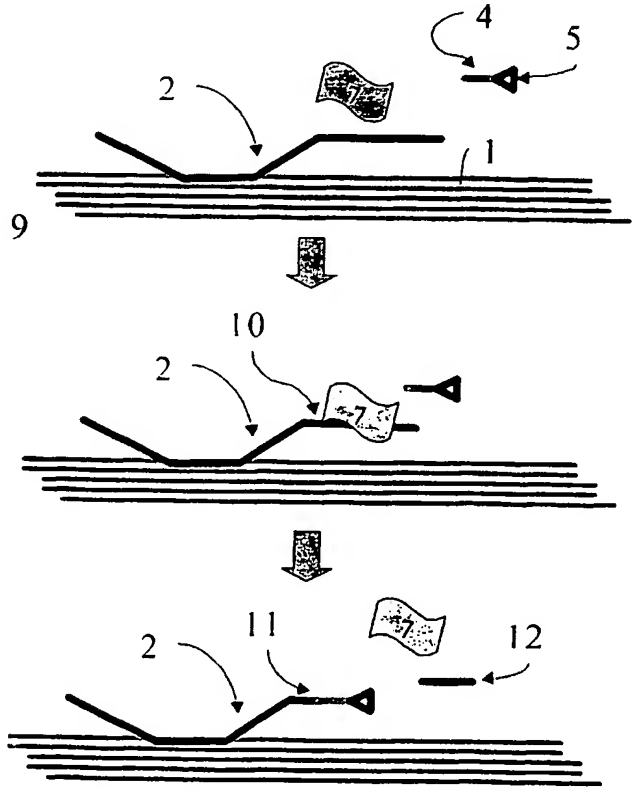
(54) Title: METHOD FOR THE MODIFICATION OF POLYMERIC CARBOHYDRATE MATERIALS



(57) Abstract: The invention makes available a method to introduce specific chemical groups onto the surface of any polymeric carbohydrate material to alter the physico-chemical properties of said material. In particular, the method comprises the controlled introduction of chemically-modified oligosaccharides into a carbohydrate polymer using a transglycosylating enzyme.

WO 03/033813 A1

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Method for the modification of polymeric carbohydrate materials

FIELD OF THE INVENTION

The present invention relates to a chemo-enzymatic method for the modification of polymeric carbohydrate materials, in particular to utilize an activated polymer interface to introduce specific chemical groups onto the surface of any polymeric carbohydrate material to alter the physico-chemical properties of said material, as well as materials produced by this method and products comprising these materials.

TECHNICAL BACKGROUND AND PRIOR ART

Virtually all cellulose materials used in the paper and board and textile industries are chemically treated to alter the surface properties of these materials, either before (e.g. wood pulp, cotton thread, etc.) or after formation of the product in its final three-dimensional form (e.g. paper sheets, corrugated cardboard, woven fabrics, etc). Treatment of cellulose materials with chemical additives at various points in the manufacturing process leads to dramatic changes in fibre surface properties. For example, carboxymethylcellulose, an anionic cellulose derivative, is added to wood pulps to increase the retention of commonly used cationic fillers and sizing agents.

Similarly, organic sizing agents such as alkyl ketene dimer and alkyl succinic anhydride are added during paper sheet formation to increase hydrophobicity and effect sheet printability. In the use of cellulose materials as packaging agents for liquids and foodstuffs, paper and cardboard are often laminated with a thermoplastic, such as polyethylene to provide an impermeable barrier to aqueous solutions. Both textiles and paper sheets are routinely dyed or printed upon, which is yet another example of surface modification. Much of current technology in security papers and packaging (bank notes, traceable documents and packages) relies upon surface treatments with specific chemicals, which can be later analysed to determine authenticity. Furthermore, cellulosic materials have great potential in the polymer industry for a wide range of applications, such as fillers, laminates and panel products, wood-polymer composites, polymer composites, alloys and blends, and cellulose derivates (cellulosics).

However, owing to some bottlenecks in their manufacture or performance, the use of such composites is still limited. In recent years, the demand has risen for surface-modified fillers that improve the properties of the virgin polymer and reduce the cost or the weight of finished products. Optimization of the interfacial bond between fibre and polymer matrix

is an important aspect with respect to optimal mechanical performance and durability of fibre reinforced composites. The quality of the fibre-matrix interface is significant for the application of natural fibres as reinforcement for plastics. Since the fibres and matrices are chemically different, strong adhesion at their interfaces is needed for an effective transfer of stress and bond distribution throughout an interface. New methods for the derivatization of cellulose are thus needed in order to improve the fibre-matrix adhesion for polymer processing, adhesives and novel composite materials.

The currently available technology for cellulose fibre surface modification by physical and chemical treatments lacks a high degree of control in the manner by which agents are introduced onto the fibre surface. A particularly serious shortcoming of direct chemical modification of cellulose is that most chemicals penetrate into the fibre structure and the chemical modifications occurring inside the fibres lead to loss of fibre structure and properties. As catalysts, enzymes are highly specific in their mode of action, and therefore offer an attractive alternative to traditional methods. In addition, the proteinaceous nature of these catalysts means that they are readily biodegradable and environmentally friendly. Furthermore, enzymes are naturally surface acting thus overcoming the problem of penetration into the fibre structure.

Degrading enzymes, which catalyse the breakdown of their substrates by the cleavage of chemical bonds, have received increasing attention over the past 15 years for the treatment of cellulose materials, most notably in the pulp and paper industry. For example, ligninases are used to improve the bleachability and brightness of pulps by removal of lignin, while xylanase treatment facilitates the removal of re-precipitated lignin during the cooking process. Similarly, cellulases are used extensively in the textile industry to effect garment finish. For example, cellulase treatment of denim has largely replaced mechanical tumbling with pumice stones to create so-called "stone-washed" effects. Cellulases are also a key ingredient in a number of laundry detergents, where they act as depilling agents by enzymatically trimming frayed cotton fibres.

Despite the widespread use of degrading enzymes in cellulose fibre modification, the use of enzymes, which operate in the opposite direction, i.e. the synthetic direction, is little developed. This is largely because comparatively little is known about the enzymes responsible for the synthesis of polysaccharides such as cellulose and hemicellulose. The majority of these enzymes, known as nucleotide sugar-dependent transferases, are cell membrane-bound, which makes their isolation and characterisation difficult. In addition, the preparation of the activated sugars is expensive. The use of nucleotide sugar-dependent transferases in cellulose fibre modification is further limited by the fact that chemical modification of the sugar ring of the natural substrates, which are ultimately

incorporated into the growing polysaccharide chain, is not tolerated. Aside transferases, glycosyl hydrolases using a retaining reaction mechanism, such as certain β-glucosidases or cellulases, can also be used for carbohydrate synthesis if water is excluded from the reaction mixture. When operated in organic solvents these enzymes catalyze transglycosylation reactions leading to formation rather than degradation of glycosidic bonds.

However, the need to use organic solvents is a significant drawback of this method. Further, retaining glycosidases can be genetically engineered to remove their catalytic nucleophile. Such an enzyme can not form a covalent enzyme-substrate intermediate required for hydrolysis but can instead catalyse condensation of appropriate acceptor and donor sugars together if the donor sugar is fluorinated to mimic the transition state of the reaction. As with the nucleotide-dependent glycosyl transferases, the drawback is the need of activated substrates, which will limit the use of the technology in large scale applications.

Thus, there is a need to develop processes for the introduction of a wide range of chemical groups with different functionalities on polymer carbohydrate materials and in particular on cellulose fibres without compromising the intactness of the fibre structure. Ideally, the process should involve one or more enzymes, which are devoid of hydrolytic or other degradative activity since this would work against any attempts to append chemical groups to the fibres.

EP 562 832 discloses a gene coding for an endo-xyloglucan transferase and suggested this gene for use in regulating the morphology of a plant. The disclosure also mentions a method of transferring xyloglucan molecules which comprises splitting a D-glucosyl linkage in a xyloglucan molecule by using an endoglucan transferase and linking the resultant reducing end of xyloglucan molecular segment to D-glucose of the non-reducing end of another xyloglucan molecule. Repeating this a number of times, xyloglucan molecules of an arbitrary structure can be constructed, which is said to be applicable to the synthesis of chimeric polysaccharides.

U.S. 5,968,813 (a continuation of PCT/DK96/00538, published as WO 97/23683) discloses a process for improving the strength properties of cellulose materials, according to which a cellulose material is contacted with a xyloglucan endotransglycosylase (XET) in an aqueous medium. The XET treatment is believed to increase cross-linking between the cellulose fibres, thus improving the strength and/or shape retention of the cellulose material.

SUMMARY OF THE INVENTION

The present invention relates to method of modifying a polymeric carbohydrate material (PCM), the method comprising a step of binding a chemical group having a desired functionality to said carbohydrate material by means of a carbohydrate linker molecule carrying the chemical group, said linker molecule is capable of binding to the PCM.

In one embodiment of the method comprises the steps of providing a carbohydrate polymer fragment (CPF) comprising a chemical group having a desired functionality, which typically could be hydrofobic, charged, reactive. The CPF is brought into contact with a soluble carbohydrate polymer (SCP) under conditions which lead to the formation of a complex between the CPF and at least a part of the SCP. The PCM is finally modified by letting the SCP bind to the PCM.

The present invention further relates to materials and compositions created be method and to materials and compositions comprising the complex of the PCM and a SCP comprising a chemical group having a desired functionality.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of modifying a polymeric carbohydrate material (PCM), the method comprising a step of binding a chemical group having a desired functionality to said carbohydrate material by means of a carbohydrate linker molecule comprising the chemical group, said carbohydrate linker molecule is capable of binding to the PCM.

An embodiment of this method is illustrated in Figure 1, showing the unmodified PCM (1), and the carbohydrate linker molecule (CLM) (2), said CLM (2) comprising at least a part of a SCP (3), and a chemical group (5) and optionally complexed with a carbohydrate polymer fragment (CPF) (4) comprising the chemical group. Because the carbohydrate linker molecule is capable of binding to the PCM, binding will occur when the PCM are brought in contact.

In another embodiment of the invention the method comprises the steps of

(i) providing a carbohydrate polymer fragment (CPF) comprising a chemical group having a desired functionality,

(ii) bringing said CPF comprising the chemical group into contact with a soluble polymeric carbohydrate (SCP) under conditions leading to the formation of a

complex consisting of said CPF comprising the chemical group, and the SCP, said CPF and SCP together forming the carbohydrate linker molecule (CLM), and

(iii) contacting said CLM with the PCM to be modified under conditions where the CLM binds to the PCM to obtain the modified polymeric carbohydrate material.

The term "polymeric carbohydrate materials" which is abbreviated "PCM" relates to a material that comprises a water-insoluble polymeric carbohydrate material and/or a water-soluble polymeric carbohydrate material. The PCM may be any material, which wholly or partly is made up of repeating units of one or more monosaccharides. Such PCMs are often composites with two or more different types of polymeric carbohydrates or a carbohyrdate polymer and another polymers such as protein. The PCM may comprise a chitin, which is a polymer of *N*-acetylglucosamine, which often forms complexes with proteins or other polysaccharides such as mannan.

The PCM may also comprise cellulose. Cellulose may be a homopolymer of β-1,4-linked glucose units. The long homopolymers of glucose (e.g. 8-15000 glucose units) stack onto one another by hydrogen bonds, thus forming an insoluble material. Such cellulose materials may be completely crystalline, or they may occur in disordered, amorphous form or they may be a mixture of the two. They may also be produced by first solubilizing the insoluble cellulose material and then regenerating it to form insoluble cellulose material of different chain organization (cellulose II).

Cellulose in the plant cell walls forms complexes with other, soluble cell wall polysaccharides such as hemicelluloses and pectin. Examples of PCMs comprising cellulose and/or cellulose/hemicellulose composites are cellulose fibres, cellulose microfibrils (whiskers), paper and pulp products and cellulose fabrics.

As will be apparent from the description and the examples, the term PCM relates to any structures in small polymers (e.g. dimensions less than one nm), large polymers (e.g. dimensions of 0.1 - 1000 nm), aggregates of polymers (e.g. dimensions of 1 - 10.000 nm), fibres (e.g. dimensions of 0.1-100.000 μm), aggregates of fibres (e.g. dimensions of 0.00001 - 1000 m).

The term "cellulosic fibre" relates to a plant cell consisting of an outer primary cell wall, which encapsulates a thicker and more complex secondary cell wall. The essential fibre component is cellulose, which is the load-bearing component of the plant cell walls. Depending on different pulping sequences, pulp fibres may or may not contain primary cell wall material. The term "cellulose microfibrils" relates to the elementrary units of cellulose

crystals produced by plants or other organisms. Cellulose microfibrils can be prepared from cellulosic plant fibres, or more easily from cultures of cellulose synthesizing bacteria such as *Acetobacter xylinum* spp.

In the context of the present invention cellulose fibres may be extracted from an annual plant such as for example flax, hemp or cereals or perennial plant such as for example cotton, poplar, birch, willow, eucalyptus, larch, pine or spruce. Cellulose microfibrils can be obtained from bacterial cultures of e.g. *Acetobacter xylinum* spp. A paper or pulp product may be any cellulose-containing material known in the art. These include, but are not limited to materials such as wood or pulp fibres, different chemical pulps, mechanical and thermomechanical pulps, fluff pulps, filter papers, fine papers, newsprint, regenerated cellulose materials, liner boards, tissue and other hygiene products, sack and Kraft papers, other packaging materials, particle boards and fibre boards as well as surfaces of solid wood products or wood and fibre composites.

Further examples of the PCM comprise polymeric carbohydrate materials used in medical applications, such as membranes, gels, beads used in diagnostics or separation technology, and membranes used in electronic applications. The fibre product in the context of the present invention may also be a new type of composite with other natural or synthetic polymers or materials as well as electronic compounds.

In the context of the present invention a cellulose fabric is any cellulose-containing fabric known in the art, such as cotton, viscose, cupro, acetate and triacetate fibres, modal, rayon, ramie, linen, Tencel® etc., or mixtures thereof, or mixtures of any of these fibres, or mixtures of any of these fibres together with synthetic fibres or wool such as mixtures of cotton and spandex (stretch-denim), Tencel® and wool, viscose and polyester, cotton and polyester, and cotton and wool.

The term "soluble carbohydrate polymers" which is abbreviated (SCP), relates to polymers comprising one or more different monosaccharides or their derivatives, which can be dissolved in aqueous or organic solvents. Examples include polysaccharides classified as hemicelluloses (those carbohydrate polymers which are not composed only of β(1-4)-linked glucose units, i.e., cellulose), pectins (polyuronic acids and esters), and starches (α (1-4)-linked polyglucose with or without α (1-6) sidechain branching). Xyloglucan, which is a polysaccharide composed of a β(1-4)-linked polyglucose backbone decorated with α(1-6) xylose residues, which themselves can be further substituted with other saccharides such as fucose and arabinose, is an example of such a SCP, specifically a hemicellulose. In a preferred embodiment the SCP is capable of binding to the PCM, e.g. via one or more hydrogen bonds, ionic interaction, one or more covalent bonds, van der Waals forces or

any combination of these. In an embodiment of the present invention the SPC may be a CPF according to the description below.

The term "carbohydrate polymer fragments" which is abbreviated "CPF" relates to molecules that may be enzymatically or chemically prepared fragments of the SCPs. Examples of such fragments comprise any number of the repeating units of said SCPs. Suitable fragments may thus contain from 2 to approximately 5000 monosaccharide units in the polymer backbone such as approximately 2-10, 4-10, 3-100, 11-15, 20-25, 26-40, 41-60, 61-100, 101-200, 201-300, 301-400, 401-500, 501-1000, 1001-2000, 2001-3000, 3001-4000 or 4001-5000 monosaccharide units. The CPF may further comprise side chains of different length and composition. Specific examples include but are not limited to xylogluco-oligosaccharides (XGO) such as of the structures described in Figure 4 or a fragment thereof, or as further modified with one or more fucosyl residues or other monosaccharides.

XGOs are commonly named according to the nomenclature system outlined in Fry et al. (1993) Physiologia Plantarum, 89, 1-3 where G represents an unsubstituted beta-glucopyranosyl residue, X represents a xylopyranosyl-alpha(1-6)-glucopyranosyl unit, L represents a galactopyranosyl-beta(1-2)-xylopyranosyl-alpha(1-6)-glucosyl unit, F represents a fucopyranosyl-alpha(1-2)-galactopyranosyl-beta(1-2)-xylopyranosyl-alpha(1-6)-glucosyl unit, among others. These various units may be connected via a beta(1-4) linkage between the glucopyranosyl units to form a beta(1-4)-glucan polysaccharide backbone. Using this nomenclature, the XGOs which are commonly isolated after endoglucanase digestion of tamarind xyloglucan are XXXG, XLXG, XXLG, and XLLG (see Figure 4). If the reducing-end glucose (G) of these oligosaccharides is in the reduced, alditol form, this unit is represented by "Gol". Thus, for example, the reduced (alditol) derivatives of the aforementioned oligosaccharides from tamarind xyloglucan are designated XXXGol, XLXGol, XXLGol, and XLLGol.

The term "carbohydrate polymer fragments" which is abbreviated "CPF" relates to molecules that may be enzymatically or chemically prepared fragments of the SCPs. Examples of such fragments comprise any number of the repeating units of said SCPs. Suitable fragments may thus contain from 2 to approximately 5000 monosaccharide units in the polymer backbone such as approximately 2-10, 4-10, 3-100, 11-15, 20-25, 26-40, 41-60, 61-100, 101-200, 201-300, 301-400, 401-500, 501-1000, 1001-2000, 2001-3000, 3001-4000 or 4001-5000 monosaccharide units. The CPF may further comprise side chains of different length and composition. Specific examples include but are not limited to xylogluco-oligosaccharides (XGO) such as of the structures described in Figure 4 or a

fragment thereof, or as further modified with one or more fucosyl residues or other monosaccharides. XGOs are commonly named according to the nomenclature system outlined in Fry et al. (1993) Physiologia Plantarum, **89**, 1-3 where G represents an unsubstituted beta-glucopyranosyl residue, X represents a xylopyranosyl-alpha(1-6)-glucopyranosyl unit, L represents a galactopyranosyl-beta(1-2)-xylopyranosyl-alpha(1-6)-glucosyl unit, F represents a fucopyranosyl-alpha(1-2)-galactopyranosyl-beta(1-2)-xylopyranosyl-alpha(1-6)-glucosyl unit, among others. Using this nomenclature, the XGOs which are commonly isolated after endoglucanase digestion of tamarind xyloglucan are XXXG, XLXG, XXLG, and XLLG (see Figure 4).

In the context of the present invention the term "chemical group" relates to any chemical radical (R-) group of potential interest for activation or modification of the insoluble polymeric carbohydrate surfaces. Activation of the insoluble polymeric carbohydrate surfaces is defined as a modification which will allow further chemical or enzymatic reactions to be carried out while modification of the surfaces is defined as a treatment which as such is sufficient to alter its functional properties.

Examples of chemical groups suitable for such activation or modification may include ionic groups (cationic, e.g. quaternary amino groups, ammonium groups, carbocations, sulfonium groups, or metal cations, etc.; anionic, e.g., alcoxides, thiolates, phosphonates, carbanions, carboxylates, boronates, sulfonates, Bunte salts, etc.; or zwitterionic, e.g., amino acids, ylides, or other combinations of anionic and cationic groups on the same molecule) or their unionised conjugate acids or bases (as appropriate), hydrophobic groups (alkyl hydrocarbons, e.g, fatty acyl or alkyl groups and unsaturated derivatives, or perfluoro alkanes; or aryl hydrocarbons, e.g., aromatic or polycyclic aromatic hydrocarbons or heterocycles), uncharged hydrophilic groups (e.g. polyethers, such as polyethylene glycol), potentially reactive groups such as those containing electrophilic atoms (e.g., carbonyl compounds, carbocations, alkyl halides, acetals, etc.), nucleophiles (e.g., nitrogen, sulfur, oxygen, carbanions, etc.), or monomers for polymerisation reactions (free radical, e.g., acrylamide, bromobutyrate, vinyl, styrene, etc.; or otherwise, e.g., nucleophilic or electrophilic reagents), chromophoric or fluorophoric groups (pigments, dyes, or optical brighteners, e.g., C.I. dyes, fluorescein, sulforhodamine, pyrene), biotin, radioactive isotopes, free-radical precursors and stable free radical moieties (e.g., TEMPO), nucleic acid sequences, amino acid sequences, proteins or protein-binding agents (e.g., affinity ligands, biotin, avidin, streptavidin, carbohydrates, antibodies, or enzyme substrates or their analogues), receptors, hormones, vitamins and drugs.

The term "carbohydrate linker molecules" which is abbreviated "CLM", relates to a molecule or complex which contains at least a part of a SCP according to the description above and a chemical group. In a preferred embodiment the CLM is capable of binding to the PCM, e.g. via one or more hydrogen bonds, ionic interaction, one or more covalent bonds, van der Waals forces or any combination of these.

Examples 9, 13, 15, 16, 18a, and 30 show the application of a chemical group, sulphorhodamine, which is chromophoric, fluorophoric, and zwitterionic, to modify cellulosic materials. Chromophoric groups are generally known as pigments, fluorophores are used as optical brighteners in textile and other applications, and ionic compounds act as retention aids in papermaking. Examples 10, 14, 17, 18b, and 21 show cellulosic fibre modification with fluorescein, which is likewise chromophoric, fluorophoric, and anionic over a wide pH range, and therefore will have applications where those groups are desired. Examples 8, 19, and 20 outline methods for the incorporation of an amino group to the fibre surface, which is cationic over a wide pH range and thus is suitable as an ion-exchange agent and can also increase retention in papermaking.

Furthermore, the amino group is intrinsically more reactive than the chemical groups already present in cellulosic fibres and can thus be used for coupling a wide range of other chemicals to the fibre surface. The incorporation of radioactivity is demonstrated in Example 11 and in the XET enzyme assay described in part "a." under that heading. Radioactivity can be used for tracer applications and fibre morphology studies. Reactions to incorporated alkyl chains are described in Examples 22, 24, and 25. In particular, Examples 24 and 25 show how alkenyl succinic anhydride, a common paper hydrophobizing agent, can be specifically coupled to the fibre surface, potentially increasing retention of this group.

Examples 23 and 26 demonstrate that non-fluorophoric aromatic groups can be coupled to the fibre; the latter Example incorporates a cinnamoyl group, which can under go polymerization reactions such as those producing polystyrene and lignin. Likewise, the bromoisobutyryl group attached as described in Example 27 is another initiator for free-radical polymerization reactions. As described in Example 31, such groups can be used to produce cellulose-based graft co-polymers, which have high quality fibre-matrix interfaces with very strong mutual adhesion but low or no detrimental effect of the fibre/cellulose structure. The incorporation of biotin in Example 28 allows the direct coupling of avidin protein conjugates to the fibre surface, which is broad in scope and can be used to introduce enzyme and protein binding activity to the fibre.

Addition of a thiol (or sulfhydryl) group allows for highly specific and, most importantly, reversible coupling of other thiols via disulfide bond formation, as described in Examples 29 and 30. A vast array of chemical groups can be specifically introduced by this procedure and subsequently removed when no longer desired. Finally, all of the Examples mentioned above demonstrate that a wide variety of amine-reactive chemical groups can be used to incorporate other functionalities, including but not limited to, sulfonyl chlorides, isothiocyanates, isocyanates, acid anhydrides, activated carboxyl compounds (even those produced *in situ*), and thioesters. Many of these chemistries can be used to carry our reactions enhancing for example the fibre-fibre bonding or the reactivity of cellulose with other materials.

The CLM may be prepared by organic or chemical synthesis and/or by using the catalytic activity of certain enzymes. An embodiment of preparing a CLM using an enzyme and an CPF is illustrated in Figure 2. The SCP (8) is contacted with an enzyme (7) and CPF (4) comprising a chemical group (5). In this embodiment the enzyme (7) cleaves the SCP and incorporates the CPF with the chemical group instead, resulting in the product CLM (2). The CLM may comprise one or more chemical groups.

In an embodiment of the present invention, the CLM may be prepared using an enzyme capable of transferring native or chemically modified mono- or oligosaccharides onto the ends of oligo- or polysaccharides. Such enzymes include but are not limited to enzymes, have high transglycosylation activity but low hydrolytic activity, glucosyl hydrolases with high inherent transglycosylation activity, enzymes, which have been biotechnically engineered to enhance their transglycosylation activity and glycosyl transferases, which use nucleotide sugars as substrates.

In an embodiment of the present invention the enzyme may be defined as any enzyme which, when assayed with a suitable glycosyl donor substrate (e.g., xyloglucan) in the presence and absence of a mono-, oligo-, or polysaccharide acceptor substrate (e.g., XGO) under appropriate conditions to maintain enzyme activity, exhibits a rate of incorporation of the acceptor substrate into the donor substrate which is at least 10% of the hydrolytic rate, such as at least 15%, 20%, 25%, 30%, 40%, 50%, 75%, 100%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, such as at least 1000% of the hydrolytic rate. The assay used for determining the rates of incorporation of the acceptor substrate into the donor substrate may be the Radiometric assay and/or the Colorimetric assay for determining enzyme activity which are described herein.

Representative examples of enzymes with naturally high transglycosylating activity include but are not limited to amylosucrases (Skov *et al* 2001. *J Biol Chem.* 276:25273-8) and

cyclodextrin glycosyltransferase (Ven der Veen *et al* 2000. *Biochim Biophys Acta*. 1543:336-360). Many glycosyl hydrolases which operate by a retaining mechanism have transglycosylation activity, which can be enhanced by the use of organic solvents. Examples of such hydrolases include some cellulases and mannanases (Kwon *et al* 2002. *Biosci Biotechnol Biochem*. 66:110-6; Harjunpaa *et al* 1999. *FEBS Lett*. 443:149-53), xylanases (Christakopoulos *et al* 1996. *Carbohydr Res*. 289:91-104) and chitinases (Sasaki *et al* 2002. *J Biochem* (Tokyo). 131:557-64). Examples of enzymes, which have been genetically engineered for increased transglycosylating activity are so called glycosynthases based on retaining glycosyl hydrolases (Meyer *et al* 2001. *Chem Biol*. 8:437-43; Fairweather *et al* 2002. *Chembiochem*. 3:866-73). Examples of these types of enzymes are used for synthesizing designer oligosaccharides for academic, industrial and potential therapeutic purposes.

Preferably an enzyme is chosen having high transglycosylating activity and most preferably also for all practical purposes low or undetectable hydrolytic or other degradative activity. Preferably no nucleotide sugars or organic solvents are required to promote the transglycosylating activity. One example of such transglycosylating enzymes is xyloglucan endotransglycosylase, an enzyme known from plants.

For example, Stephen C. Fry *et al.* suggest in Biochem. J *15 (1992) 282,* p. 821-828 that XET is responsible for cutting and rejoining intermicrofibrillar xyloglucan chains and that XET thus causes the wall-loosening required for plant cell expansion. XET is believed to be present in all plants, in particular in all land plants. XET has been extracted from dicotyledons, monocotyledons, in particular graminaceous monocotyledons and liliaceous monocotyledons, and also from a moss and a liverwort. XET may be obtained from a plant as described in Example 1 (cauliflower) and in Example 5 (hybrid aspen cell suspension culture), or it may be obtained as described in Fry *et al. (supra).*

Alternatively, the transglycosylating enzyme is produced by aerobic cultivation of a host organism transformed with the genetic information encoding the transglycosylating enzyme. The host organism can be a plant in particular tobacco, maize or hybrid aspen, fungi in particular yeasts such as *Pichia pastoris* or *Saccharomyces cerevisiae,* filamentous fungi such as *Trichoderma reesei* or *Aspergilli*, containing the appropriate genetic information required for heterologous protein expression in the host in question. Such transformants can be prepared and cultivated by methods known in the art.

Genes: The gene encoding the transglycosylating enzyme can be obtained from nature, from an organism expressing a suitable transglycosylating enzyme, e.g. a plant or a micro-organism. The gene can also be constructed by means of genetic engineering, based on

available knowledge of naturally occurring enzymes, and modified by deletion, substitution or addition of sequence information, such as coding regions and promotors.
The XET gene may for example be obtained from cauliflower (Example 3), from hybrid aspen (Example 4) or as disclosed in EP 562 836, the disclosure of which is hereby incorporated by reference.

Host cells: The host cells comprising the resulting DNA construct may be obtained using methods known to a skilled person in this field.

The host cell is preferably a eukaryotic cell, in particular a plant cell such as poplar or tobacco cell suspension or tissue culture, or the leaves or seeds of said plants and similar plants. The plant cells can be transformed by *Agrobacterium* mediated gene transfer or by using a particle gun in a manner known *per se*. The host cell can also be yeast or filamentous fungal cell or a bacterial cell. In particular, the cell may belong to a species of *Trichoderma,* preferably *Trichoderma harzianum* or *Trichoderma reesei,* or a species of *Aspergillus,* most preferably *Aspergillus oryzae* or *Aspergillus niger.* Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se.

The use of *Aspergillus* as a host micro-organism has been described inter alia in EP 238 023 (Novo Nordisk A/S), and the use of *Trichoderma* has been described inter alia in EP0244234 A2 04-11-1987 [1987/45], EP0244234 A3 12-10-1988 [1988/41], EP0244234 B1 21-07-1993 [1993/29], EP0244234 B2 07-11-2001 [2001/45]; the contents of which are hereby incorporated by reference. The host cell may also be a yeast cell, e.g., a strain of *Saccharomyces,* in particular *Saccharomyces cerevisiae,* or a strain of *Pichia* sp. such as *Pichia pastoris* or *Kluyveromyces* sp., such as *Kluyveromyces lactis.* The host can also be bacterium, such as for example gram positive bacterium *Bacillus subtilis,* or gram negative bacteria such as *E. coli.* The transformation of the bacteria may, for instance, be effected by protoplast transformation or by using competent cells in a manner known *per se.*

According to the invention, a transglycosylating enzyme may be obtained from a dicotyledon or a monocotyledon, in particular a dicotyledon selected from the group consisting of the following families of plants; cauliflower, soy bean, tomato, potato, rape, sunflower, cotton, tobacco and poplar, or a monocotyledon selected from the group consisting of wheat, rice, corn and sugar cane. Examples of such enzymes is any enzyme encoded by one of the sequences SEQ.ID.NO. 1, 2, 3, or by a functional homologue thereof. By functional homologue is herein intended a sequence, exhibiting homology with

enzyme encoded by one of the sequences SEQ.ID.NO. 1, 2, 3, said homology is at least of 50% such as at least 60%, 70%, 80%, 90%, 95%, 99% or 100% percent.

The functional homologue may alternatively be an enzyme encoded from a nucleic acid sequence, said nucleic acid sequence having a homology with at least one of the sequences in SEQ.ID.NO. 1, 2, 3 of at least 50% such as at least 60%, 70%, 80%, 90%, 95%, 99% or 100% percent.

In the present context, the term "homology " indicates a quantitative measure of the degree of homology between two amino acid sequences of equal length or between two nucleotide sequences of equal length. If the two sequences to be compared are not of equal length, they must be aligned to the best possible fit. The sequence identity can be calculated as $\frac{(N_{ref}-N_{dif})100}{N_{ref}}$, wherein Ndif is the total number of non-identical residues in the two sequences when aligned, and wherein Nref is the number of residues in one of the sequences. Hence, the DNA sequence AGTCAGTC will have a sequence identity of 75% with the sequence AATCAATC (Ndif=2 and Nref=8). A gap is counted as non-identity of the specific residue(s), i.e. the DNA sequence AGTGTC will have a sequence identity of 75% with the DNA sequence AGTCAGTC (Ndif=2 and Nref=8). Sequence identity can alternatively be calculated by the BLAST program, e.g. the BLASTP program (Pearson & Lipman (1988) (www.ncbi.nlm.nih.gov/cgi-bin/BLAST). In one aspect of the invention, alignment is performed with the global align algorithm with default parameters as described by Huang & Miller (1991), available at http://www.ch.embnet.org/software/LALIGN_form.html.

Alternatively the enzyme may be an enzyme which exhibits lower sequence homology with the said sequences but has been engineered to have transglycosylating activity.

The present inventors have based the invention *inter alia* on the facts that xyloglucan, naturally present in the primary cell walls of plant fibres, is able to make strong hydrogen bonds to cellulose, and that endogenous XET activity of plants results in the incorporation of radioactive and fluorescent XGOs to the xyloglucan component of plant cell suspension cultures (see Biochem J. 279, 1991, p.529-535 and Plant Cell Physiol 40, 1999, p 1172 - 1176).

The inventors have then found that isolated xyloglucan polymers can be chemically and/or enzymatically modified to contain a wide range of different chemical groups and that such chemically modified xyloglucan polymers can be used as an interface for introducing new chemical groups onto the cellulosic fibre surfaces. A significant advantage of the method is

that the use of such interface polymers avoids subsequent loss of fibre structure and performance otherwise commonly encountered with direct chemical modification of cellulose. The inventors have found that that a transglycosylating enzyme, such as XET, can use, as acceptor sugars, oligosaccharides containing different types of chemical groups.

Since water does not compete as a transglycosylation acceptor for such enzymes, they can be used in aqueous solutions to efficiently incorporate said chemical groups onto the interface polymers, such as xyloglucan, either in solution or when bound to another polymeric material such as cellulose. Further, the inventors have found that xyloglucan, even when chemically modified, binds tightly to the surface of the cellulose, and that the chemical groups introduced are, even when attached to the porous surfaces of cellulosic materials via XG, nevertheless accessible for further chemical reactions.
The inventors have then found that by adding a transglycosylating enzyme and chemically modified CPFs to SCPs, it was surprisingly possible to attach many different new chemically chemical groups with a desired functionality onto PCM surfaces with a high yield.

The PCM to be modified may be derived from a plant selected from the group consisting of a monocotyledonous plant, such as a plant of the family *Gramineae,* and a dicotyledonous plant such as a plant is selected from the group consisting of angiospermous plants (hardwoods), coniferous plants (softwoods) and plants belonging to the *Gossypium* family.

The PCM may be in the form of cellulosic plant fibres or in the form of cellulosic microfibrils derived from cellulosic plant fibres or from a bacterium.

The SCP may form a part of the PCM to be modified, thus the step of incorporating the CPF comprising a chemical group with a desired functionality in the SCP, e.g. using an enzyme, may be performed directly on the PCM-SCP complex. The principle is illustrated in Figure 3. In upper part of Figure 3 one sees the PCM-SCP complex (9) comprises the PCM (1) and the SCP (2), the enzyme (7) and a CPF (4) comprising a chemical group with a desired functionality (5). In the middle part of Figure 3 the enzyme (7) binds to the SCP (2) of the PCM-SCP complex (9) and may form an intermediate complex (10). In the process leading to the lower part of Figure 3, the enzyme (7) cleaves the SCP (2) and incorporates (12) the CPF (4) comprising a chemical group with a desired functionality (5). (12) is the SCP fragment which was cleaves off the SCP.

Alternatively, the SCP needs not to be associated with the PCM to be modified. In the latter case the SCP may be modified to comprise the chemical group and product of the SCP modification, CLM, is then contacted with the PCM. Alternatively, SCP is first contacted

with SCP-less PCM. When the SCP-PCM complex has been formed, the step of incorporating the CPF comprising a chemical group with a desired functionality the SCP, e.g. using an enzyme, may be performed directly on the PCM-SCP complex.

It is possible to modify the PCM with a mixture of chemical groups, e.g. by performing the methods described herein in sequence and/or by using a mixture of CLMs comprising different chemical groups and binding the mixture of CLMs comprising different chemical groups to the PCM in one process step.

Both SCP and/or CPF may contain the chemical group.

CPF may be derived from xyloglucan and may contain from 3 to about 100 including from 4 to 10 polymer backbone monosaccharide units.

In an embodiment CPF comprising the chemical group is brought into contact with the soluble polymeric carbohydrate (SCP) in the presence of an enzyme that is capable of promoting the formation of the complex consisting of said CPF comprising the chemical group, and at least a part of the SCP. The enzyme may be capable of transferring native or chemically modified mono- or oligosaccharides onto an oligo- and/or polysaccharide. In an embodiment the enzyme may be an enzyme having transglycosylation activity.

In another embodiment, the enzyme exhibits a rate of incorporation of the acceptor substrate into the donor substrate which is at least 10% of the hydrolytic rate, such as at least 15%, 20%, 25%, 30%, 40%, 50% or 75%, such as at least 100%, when assayed with a suitable glycosyl donor substrate in the presence and absence of a mono-, oligo-, or polysaccharide acceptor substrate under appropriate conditions to maintain enzyme activity. The glycosyl donor substrate may be a xyloglucan and the acceptor substrate may be a xyloglucan-oligosaccharide.

The assay for evaluation the rate of incorporation of the acceptor substrate into the donor substrate be an assay consists of the following steps

i) incubating 0.1 mg xyloglucan, 0.1 mg xyloglucan oligosaccharides (mixture of XXXG, XLXG, XXLG, and XLLG; 15:7:32:46 weight ratio) in 200 µL 40 mM citrate buffer pH 5.5 for 30 minutes at 30 °C.

ii) stopping the reaction with 100 µL 1M HCl,

iii) the ionic strength was adjusted by adding 800 µL 20% Na_2SO_4 and 200 µL of an I_2 (0.5 % I_2, 1% KI, w/w) solution

iv) measuring the absorbance was measured at 620 nm

v) performing the steps i)-iv) without adding the xyloglucan oligosaccharides (XGO) of step i)

vi) calculating the absorbance increase in percent between from the incubation with XGO to the incubation without XGO.

The enzyme may be selected from the group consisting of a transglycosylase, a glycosyl hydrolase, a glycosyl transferase. The enzyme may be a wild type enzyme or a functionally and/or structurally modified enzyme derived from such wild type enzyme. In an embodiment the enzyme is a xyloglucan endotransglycosylase (XET, EC 2.4.1.207).

The enzyme having transglycosylation activity may be derived from a plant including a plant belonging to the family *Brassica* and a plant of a *Populus* species or may be produced produced recombinantly.

In an embodiment, the chemical group having a desired functionality may be selected from the group consisting of an ionic group, a hydrophobic group, an uncharged hydrophilic group, a reactive group, a nucleophile, a polymerisable monomer, a chromophoric group, a fluorophoric group, biotin, a radioactive isotope, a free-radical precursor, a stable free radical moiety, a protein and a protein binding agent.

An embodiment of the present invention relates to a method wherein the obtained modified PCM has, relative to the non-modified PCM, altered surface properties, such as altered strength properties, altered surface tension, altered water repellence properties, altered reactivity, altered optical properties or combinations of these.

Another aspect of the invention is a modified polymeric carbohydrate material (mPCM) obtainable by the method of any of the methods described herein, the material having bound thereto chemical groups having a desired functionality, said binding is mediated by a carbohydrate linker molecule that is capable of binding to the PCM. The mPCM may be in the form of cellulosic plant fibres or cellulosic microfibrils derived from cellulosic plant fibres or from a bacterium.

The chemical groups of the mPCM may be reactive groups capable of binding other functional groups and the mPCM may have bound thereto two or more different types of chemical groups.

Another aspect of the invention is a composite material comprising the materials described herein.

The mPCMs or the composite materials thereof may be used in manufacturing of paper sheets, corrugated cardboard, woven fabrics, auxiliary agents in a diagnostic or chemical assay or process, packaging agents for liquids and foodstuffs, paper and cardboards which are often laminated with a thermoplastic, such as polyethylene to provide an impermeable barrier to aqueous solutions, textiles and security papers, bank notes, traceable documents fillers, laminates and panel products, wood-polymer composites, polymer composites, alloys and blends, and cellulose derivates (cellulosics).

According to the present invention, new chemical groups can be added to PCM containing an inherent suitable SCP by using the transglycosylating enzyme to couple the chemically modified CPFs to the SCP contained in the cellulose materials. In the present context the term "inherent" means that the PCM comprises a SCP prior to the modification. According to the present invention, new chemical groups can also be added to PCM not containing inherent SCP by first using the transglycosylating enzyme to couple the chemically modified CPFs to the SCP in solution followed by sorption of the modified SCP onto the PCM.

According to a specific embodiment of the present invention, new chemical groups can be added to cellulose materials not containing inherent xyloglucan by first using the XET enzyme to couple the chemically modified XGOs to xyloglucan (XG) in solution followed by sorption of the modified XG onto the cellulose materials.

According to the present invention, a PCM is given altered surface chemistry and/or improved chemical reactivity after treatment with chemically modified CPFs, which are coupled to a SCP using the transglycosylating enzyme. The SCPs carrying the chemically reactive groups will bind tightly to the PCM surfaces thus maintaining the chemical reactivity of said surfaces. The chemical reactivity *per se* or when modified by further chemical and/or polymerization reactions influences the surface properties of the PCM. Moreover, the density of the chemically reactive groups is controlled by altering the concentrations of the transglycosylating enzyme and/or the CPFs and/or the reaction time, as shown in Examples 14a and 14b. The surface properties may be measured by any method known in the art as shown in the attached examples, e.g., Example 16b.

In nature, transglycosylating enzymes, such as the XET enzyme, function *in vivo*, in the living plant, so the enzyme is clearly able to work in an aqueous environment. The method according to the invention may thus be carried out in an aqueous solution, or it may be carried out in water in the presence of certain components such as a buffer and/or a wetting agent and/or a stabiliser and/or a polymer and/or an organic component reducing the water activity such as DMSO.

The buffer may suitably be a phosphate, borate, citrate, acetate, adipate, triethanolamine, monoethanolamine, diethanolamine, carbonate (especially alkali metal or alkaline earth metal, in particular sodium or potassium carbonate, or ammonium and HCl salts), diamine, especially diaminoethane, imidazole, Tris, or amino acid buffer.
The wetting agent serves to improve the wettability of the PCM. The wetting agent is preferably of a non-ionic surfactant type. The stabiliser may be an agent stabilising the enzyme.

It will generally be appropriate to incubate the reaction medium, e.g. comprising the PCM the CLM and optionally one or more components selected from group a SPC which may or may not comprise a chemical group, a CPF comprising a chemical groups and an enzyme) for a period of at least a few minutes, depending on the reaction conditions. An incubation time of about one minute to 20 hours, such as approximately 2-5 minutes, 5-7 minutes, 7-10 minutes, 10-15 minutes, 15-20 minutes, 20-30 minutes, 30-40 minutes, 40-60 minutes, 1-2 hours, 2-4 hours, 4-6 hours, 6-8 hours, 8-10 hours, 10-12 hours, 12-14 hours, 14-16 hours, 16-18 hours or 18-20 hours, will generally be suitable. In particular an incubation time of from 30 minutes to 10 hours will often be preferred. The incubation time is preferably controlled with a time interval narrower than +/- 5 hours, such as narrower than +/- 2 hours, +/- 1 hour, +/- 45 minutes, +/- 30 minutes, +/- 15 minutes, +/- 10, minutes, +/- 5 minutes, +/- 2 minutes, +/- 1 minutes, +/- 30 seconds, +/- 10 seconds, +/- 1 second, +/- 0,1 seconds or +/- 0,01 seconds.

The temperature of the reaction medium in the process of the invention may suitably be in the range of -5 - 100 °C, such as 0 - 5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-33, 33-36, 36-38, 38-40, 40-50, 50-60, 60-70, 70-80, 80-90, or 90-100 °C. In embodiments where the reaction mixture comprises an enzyme the temperature of reaction mixture during incubation should preferably be in near the temperature that creates the optimal turnover during the incubation. Preferably temperature should be less than 10 °C from the temperature that creates the optimal turnover during the incubation, such as less than 10 °C, 8 °C, 6 °C, 4 °C, 2 °C, 1 °C, 0.5 °C or 0.1 °C.

For binding the modified or unmodified SCP to the PCM, it will generally be appropriate to incubate the mixture for a period of at least a few minutes, depending on the reaction conditions. An incubation time of about one minute to 48 hours will generally be suitable, in particular an incubation time of from 30 minutes to 10 hours will often be preferred. The incubation solution may suitably be buffered in the pH range 2-11, preferably pH 5-8, with a buffer concentration between 0 and 5 M, preferably 0.0-0.1 M. The temperature of the reaction medium in this process may suitably be in the range of 10-100 oC, depending on the stability of the individual components in the mixture.

The invention will now be described in further details with reference to the accompanying drawings wherein:

Figure 1 illustrates the principle of modifying a polymeric carbohydrate material,

Figure 2 illustrates the principle of incorporating a soluble carbohydrate polymer comprising a chemical group in an polymeric carbohydrate material,

Figure 3 illustrates the principle of modifying PCM which comprises SCP prior to the modification,

Figure 4 shows examples of xyloglucan oligosaccharide structures (XGO-7 (XXXG), XGO-8 (X_{LXG}, XXLG) and XGO-9 (XLLG)),

Figure 5 illustrates the time dependence of XG-FITC production,

Figure 6 illustrates the dependence of XG-FITC production on the amount of enzyme in the reaction,

Figure 7 shows a confocal fluorescence microscopy image of XG-FITC-treated paper,

Figure 8 shows a photo of incorporation of fluorescein into paper treated with XG-NH_2,

Figure 9 illustrates the relative amounts of amino groups present on the surface of XG-NH_2-modified cellulosic paper following treatment with various amino-reactive reagents,

Figure 10 illustrates the reactivity of paper with and without modification by XG-NH_2 toward FITC, and

Figure 11 illustrates the reaction of thiolated paper with sulforhodamine methanethiosulfonate.

EXAMPLES

Materials and Methods

Determination of enzyme activity and in particular XET activity

a. *Radiometric assay*

The present inventors developed a modified assay method similar to that of Steele, N. et al. (Phytochemistry, 2000,54,667-680) and this was used as follows. [1-^{3}H]-XLLGol (300 µl, 0.36 µmol in H_2O) was added to non-radioactive xgo-9 alditol (700 µl, 8.6 µmol) in 50 mM citrate phosphate buffer pH 5.5. When used in assay this stock was diluted in buffer to a concentration of 2.24 µmol/ml (3.1 mg/ml). Radioactive XLLGol stock (10 µl, 2.24 µmol/ml) was added to xyloglucan (10 µL, 3.0 mg/ml in buffer). Diluted enzyme solution (10 µl) was added and reaction mixture was incubated at 25° C for 30 min. The reaction was then stopped with a 50% solution of formic acid in water (20 µL). The reaction mixture (40 µL) was dried onto rounds of Whatman 3MM chromatography paper (diameter 20 mm). The rounds were washed for 4 h under running water, dried in a 65° C oven, and analysed for radioactive incorporation in scintillation vials containing Ready-safe scintillation cocktail (6 ml, Beckman Coulter AB, Bromma, Sweden) with a Packard Tricarb 1500 scintillation counter. There was no elution of radioactivity from paper into scintillation liquid. Blanks where measured by adding acid to reaction before enzyme, control of total added radioactivity was measured by not washing the control paper rounds. A measure of how filter paper affected the assay was obtained by comparing controls with scintillation counts from control mixture without filter paper.

b. Colorimetric assay:

The enzyme activity was measured according to a modified protocol based upon that of Sulová et al. (1995) *Anal. Biochem.* **229**, 80-85. XET was incubated with 0.1 mg xyloglucan, 0.1 mg xyloglucan oligosaccharides (mixture of XXXG, XLXG, XXLG, and XLLG; 15:7:32:46) in 200 µL 40 mM citrate buffer pH 5.5 for 30 minutes at 30 °C. The assay was stopped with 100 µL 1M HCl, the ionic strength was adjusted with 800 µL 20% Na_2SO_4 and 200 µL of an I_2 (0.5 % I_2, 1% KI, w/w) solution was added. The absorbance was measured at 620 nm. For the purposes of this document, one unit of enzyme activity is defined as 0.1 units of absorbance change (after correction for background hydrolysis) over 30 min.

EXAMPLE 1

Extraction of XET from cauliflower

The extraction of cauliflower was prepared by homogenizing the cauliflower florets in ice-cold citrate buffer (0.35 M, pH 5.5 containing 10 mM $CaCl_2$), and filtering the mixture through miracloth. The filtrate was diluted with ultrapure water (18 MΩ.cm) until the conductivity of the solution was the same as that of 0.1 M ammonium acetate buffer pH 5.5. The solution was then gently stirred with SP-Fast Flow cation exchanger (Amersham Biosciences, Sweden) for 1 hour at 4°C. The SP-FF gel was collected on a glass frit filter and was washed with 0.1 M ammonium acetate, pH 5.5, until the filtrate was clear. The gel was packed into a column and bound proteins were eluted with a linear gradient of 0 to 1.0 M NaCl in 0.1 M ammonium acetate, pH 5.5, over 10 column volumes. Fractions containing XET activity were pooled and mixed with ammonium sulfate (1 M). The sample was applied to a Resource-ISO column (1 ml, Amersham Biosciences, Sweden) and then eluted by a linear gradient of 1.0 M to 0 ammonium sulfate in ammonium acetate, pH 5.5, over 20 column volumes. Fractions containing XET activity were pooled and analyzed for purity by SDS-PAGE and silver staining. The gel showed only a single band that was confirmed to be XET by immunoblotting.

EXAMPLE 2

Extraction of XET from the cell suspension culture of hybrid aspen, *Populus tremula x tremuloides Mich.*

Poplar XET was extracted by homogenizing material from a granular cell culture in ice-cold citrate buffer (0.35 M, pH 5.5 containing 10 mM $CaCl_2$), stirring the mixture for 2 hours at 4°C, and filtering it through miracloth. The filtrate was diluted with ultrapure water (18 MΩ.cm) until the conductivity of the solution was the same as that of 0.1 M ammonium acetate buffer pH 5.5. The solution was then gently stirred with SP-Fast Flow cation exchanger (Amersham Biosciences, Sweden) for 1 hour at 4°C. The SP-Trisacryl gel was collected and washed with 0.1 M ammonium acetate, pH 5.5 through a glass frit filter until the filtrate was clear. The gel was packed into a column and bound proteins were eluted with a linear gradient of 0.0 to 1.0 M NaCl in 0.1 M ammonium acetate, pH 5.5, over 10 column volumes. Fractions containing XET activity were pooled, buffer-exchanged to 0.1 M ammonium acetate, pH 5.5, on a Sephadex G-25 size-exclusion column, and loaded onto a Resource S cation exchange column (1 ml, Pharmacia). The bound proteins were

eluted with a linear gradient of 0.0 to 1.0 M NaCl in 0.1 M ammonium acetate, pH 5.5, over 10 column volumes. Fractions containing XET activity were pooled, applied to a Sephacryl S200 column (120 ml, Amersham Biosciences, Sweden), and eluted with 2 column volumes of 0.1 M ammonium acetate, pH 5.5. Fractions corresponding to the last peak, which contained the highest amount of XET activity, were pooled and applied to the Resource S column (1 ml, Amersham Biosciences, Sweden). Fractions were then eluted with a linear gradient of 0.0 to 0.5 M NaCl in 0.1 M ammonium acetate, pH 5.5, in a volume corresponding to over 10 column volumes. Fractions containing XET activity were pooled and shown to be homogeneous by SDS-PAGE.

EXAMPLE 3

Purification of recombinant XET from *Pichia pastoris* cultivation

Cells from *Pichia pastoris* cultures transformed with the genetic material encoding XET (see examples 4. and 5.) generally showed the highest XET activity in the culture medium after 3 days of methanol induction. These yeast cells were harvested by centrifugation and the culture media were further filtrated through a 0.45 μm filter and then concentrated and desalted by ultra-filtration. The XET was purified by two step cation exchange chromatography. The concentrated culture filtrate (in a buffer of 0.1 M ammonium acetate pH 5.5) was first applied to an SP-trisacryl column and then eluted by a linear gradient of 0 to 1 M NaCl in 0.1 M ammonium acetate pH 5.5. The fractions containing XET activity were pooled desalted to 0.1 M ammonium acetate pH 5.5, and applied to a Resource S column, subsequently eluted by the same salt linear gradient as used in the first step cation exchange chromatography. The homogeneity of the protein was examined by SDS-PAGE and silver staining. Only a single band with a molecular weight about 32 kDa appeared, and this was confirmed by immunoblotting to be XET. The protocol was shown to be successful for expression of all the sequences SEQ.ID.NO.1,2,3, encoding different isoenzymes of XET.

EXAMPLE 4

Isolation of the gene coding for the XET from cauliflower

cDNA corresponding to the XET gene was isolated by extraction of RNA by grinding fresh cauliflower tissues under liquid nitrogen (N_2) and lysing the cells under denaturing conditions. The lysed cell sample was then centrifuged through a QIA Shredder column to remove the insoluble material. The RNA was subsequently selectively bound to an RNAeasy membrane, washed with buffer and finally eluted in water. The XET cDNA was prepared

using a two step Polymerase Chain Reaction (PCR) according to protocol known in the art. The first strand of cDNA was synthesized using 1 μg of RNA and an oligo $dT_{(18)}$ primer with reverse transcriptase at 55°C for 1 hour. The templates for the degenerate primers for the specific PCR reaction were obtained by N-terminal sequencing of the cauliflower XET protein indicating a sequence

I P P R K A I D V P F G R N Y.

The primer sequences of all the primers used are shown in Table 1. The reverse primer CFXETR1, and the nested primers CFXET F1 were used for a two-step nested PCR resulting in a PCR product with the correct molecular weight and sequence corresponding to XET in the glycosyl hydrolase / transglycosylase family 16. The full-length cDNA was then amplified using a series of degenerate nested primers (Table 1) followed by sequence determination of the full-length cDNA (SEQ.ID.NO.1).

Table 1. Primers used in the examples

CFXETFI	AARGCNATHGAYGTNCCNTTYGG
CFXETF2	CCNCCNAGRAARGCNATHGAYGT
CFXETRI	AAYTCRAARTCDATYTCRTCRTGYTC
CFXET -5r-I	TGCAGTGACGACCCCAGCGGT A TC
CFXET -5r-2	CAGCGGTATCACCAGCCGGCAG
CFXET -3r-I	CTGCCGGCTGGTGATACCGCTG
CFXET -3r-2	GAT ACCGCTGGGGTCGTCACTGCA
5'RACEOUTER	GCTGATGGCGATGAATGAACACTG
5'RACEINNER	CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG
3'RACEOUTER	GCGAGCACAGAATTAATACGACT
3'RACEINNER	CGAGGATCCGAATTAATACGACTCACTATAGG
CF FL-FI	AACATCATTCATCATCATCACCATCACC
CF FL-F2	CATCACCATCACCATCACCATAACATCT
CF FL RI	TGAACAGAAGCATAATACTCATAATAATCCGG
CF FL R2	CATAATAATCCGGTTCATTGAAAGTTTCG

IUB-Code for mixed bases: M=A+C, R=A+G, W=A+T, S=G+C, Y=C+T, V=A+G+C, H=A+C+T, B=G+T+C,N=A+G+C+T,K=G+T.

The primers CFXETFI, CFXETF2 and CFXETRI are gene-specific degenerate primers for the RT PCR. CFXET-5r-I, CFXET-5r-2, CFXET-3r-I and CFXET-3r-2 are gene-specific internal primers while 5'RACE OUTER, 5'RACE INNER, 3'RACE OUTER and 3'RACE INNER are complementary primers to the adapters of the RLM-RACE. CF -FL-FI, CF -FL-F2, CF -FL-RI are CF -FL-R2 gene-specific primers for the amplification of the full-length cDNA.

EXAMPLE 5

Isolation of the gene coding for the XET from hybrid aspen

The cDNA coding for a poplar XET was isolated for instance from a cambial EST library of hybrid aspen, constructed as described in Hertzberg *et al*, 1998. Annotation of the library revealed three sequences corresponding to XET-like enzymes. Full-length sequencing of one of the clones revealed that it contained a cDNA copy of a full-length XET enzyme designated XET16A (SEQ.ID.NO.3) while another clone corresponded to a second full-length XET enzyme designated XET16C (SEQ.ID.NO.2).

EXAMPLE 6

Extraction of xyloglucan from tamarind kernel powder

The method of Edwards *et al.* (Planta 1985, 163,133-140) was modified as follows. $NaBH_4$ (0.75 g) was dissolved in 2.0 M NaOH (1.5 L). De-oiled tamarind kernel powder (30 g) was added to the solution slowly with vigorous stirring (paddle stirrer) to avoid clumping. The mixture was then heated to 90°C and held at that temperature for 1 hour with continuous stirring. After partial cooling, solids were filtered off through glass fibres and discarded. After further cooling, the filtrate was acidified by slow addition of glacial acetic acid (300 ml), followed by slow addition of EtOH (3.0 l) to precipitate xyloglucan as a colorless, gelatinous mass. The solids were collected by filtering through a cotton towel and the filtrate subsequently discarded. The xyloglucan was then dissolved in pure water (1.5 L, 18 MΩ.cm) with gentle heating and re-precipitated by the slow addition of EtOH (3.0 L). The solid mass was recollected by filtration through a cotton towel, which was wrung by hand to liberate excess filtrate. The solids were subsequently dried under reduced pressure (oil pump) and ground in a household coffee grinder (Braun) to yield a fine powder (17 g).

EXAMPLE 7

Endoglucanase mediated production of xyloglucan oligosaccharides

Xyloglucan (3 g) was dissolved in 200 ml purified water (18 MΩ.cm) at 50°C with vigorous stirring. Upon cooling to 30°C, cellulase (30 mg, 4 U/mg, from *T. reesei*, Fluka) was added and the solution maintained at that temperature overnight. Activated carbon (3 g) was then added, and the mixture stirred for 15 min. Following the addition of acetonitrile (200 ml), the mixture was filtered through a pad of celite on glass fibre filter paper (Whatman

GF/A). The filtrate was then concentrated *in vacuo* (water aspirator) and the residual solvent was removed with a high vacuum (oil) pump. The mixture of xylogluco-oligosaccharides (XXXG, XLXG, XXLG, and XLLG in the molar ratio 15:7:32:46 as determined by high performance anion exchange chromatography with pulsed amperometric detection, HPAEC-PAD) was fractionated by semi-preparative HPLC on an Amide-80 column (TosoHaas, 21.5 mm x 300 mm, eluent 55:45 acetonitrile-water) when required. XLXG and XXLG were not resolvable under these conditions. Electrospray ionization mass spectrometry (Micromass Q-TOF2) was used to confirm the identity of the oligosaccharides.

Xyloglucan (3 g) was dissolved in 200 ml pure water (18 MΩ.cm) at 50°C with vigorous stirring. Upon cooling to 30°C, cellulase (30 mg, 4 U/mg, from *Trichoderma reesei*, Fluka) was added and the solution maintained at that temperature overnight. Beta-galactosidase (150 mg, 9 U/mg against lactose, from *Apergillus oryzae*, Sigma G-5160) was then added and the solution stirred for 1 h at room temperature. The solution was boiled for 3 min, followed by rapid cooling prior to the addition of activated carbon (3 g). The mixture was then stirred 15 min at room temperature. Following the addition of acetonitrile (200 ml), the mixture was filtered through a pad of celite on glass fibre filter paper (Whatman GF/A). The filtrate was then concentrated *in vacuo* (water aspirator) and the residual solvent was removed with a high vacuum (oil) pump. The mixture of xylogluco-oligosaccharides was then fractionated by semi-preparative HPLC on an Amide-80 column (TosoHaas, 21.5 mm x 300 mm, eluent 55:45 acetonitrile-water). Electrospray ionization mass spectrometry (Micromass Q-TOF2) was used to confirm the identity of the oligosaccharides.

EXAMPLE 8

Preparation of aminoalditol derivatives of xylogluco-oligosaccharides (XGO-NH_2)

Xylogluco-oligosaccharides (2.4 g, 1.9 mmol, mixture of XXXG, XLXG, XXLG, and XLLG) were dissolved in saturated ammonium hydrogencarbonate solution (50 ml). Sodium cyanoborohydride (2.4 g, 38 mmol) was then added, and the reaction stirred at room temperature in the dark. After seven days, the reaction was filtered and acetic acid was added until the solution reached pH 2. After concentration *in vacuo*, the crude product was redissolved in 75 ml water and applied in ten portions to a P2 column (Bio-rad, Bio-Gel P2, 5cm x 22cm). Fractions from each column run, which contained XGO-NH_2 and exhibited low conductivity, were pooled and concentrated to dryness (yield 1.31 g, 51%). Electrospray ionization mass spectrometry (Micromass Q-TOF2) was used to confirm the identity of the modified oligosaccharides.

EXAMPLE 9

Preparation of sulforhodamine derivatives of xylogluco-oligosaccharides (XGO-SR)

XGO aminoalditols (XGO-NH_2, 0.5 g, 0.4 mM, mixture of XXXG-NH_2, XLXG-NH_2, XXLG-NH_2, and XLLG-NH_2) were dissolved in 3% aqueous sodium tetraborate (30 ml). Sulforhodamine B acid chloride (192 mg, 0.3 mM, Fluka 86186) was dissolved in dimethyl formamide (DMF, 1 ml) and added dropwise to the stirred solution. The reaction was monitored by TLC (5:4:1 chloroform: methanol: water) and concentrated to dryness after seven days. The crude product was purified by flash chromatography of silica gel (stepwise elution with 55:45:5 and 5:4:1 chloroform: methanol: water). To remove trace amounts of silica from the product, the material was loaded onto a reverse phase chromatography column (Supelclean ENVI-18 SPE tube, 6 ml, Supelco, Bellefonte, PA, U.S.A.) and was eluted by a stepwise gradient of de-ionised water, 10% aqueous acetonitrile, and 20% aqueous acetonitrile (yield: 20 mg, 2.7%).

EXAMPLE 10

Preparation of fluorescein derivatives of xylogluco-oligosaccharides (XGO-FITC)

Fluorescein isothiocyanate Isomer I (FITC, 12 mg, 0.03 mmol, Fluka 46952) was added to a solution of XGO aminoalditols (XGO-NH_2, 45 mg, 0.036 mmol, Mixture of XXXG-NH_2, XLXG- NH_2, XXLG-NH_2, and XLLG-NH_2) in sodium bicarbonate buffer (100 mM, pH 9.0, 20 ml). The reaction was monitored by TLC (70:30:1 acetonitrile:water:acetic acid) and concentrated to dryness *in vacuo* after stirring for 24 h at room temperature. The crude product was redissolved in 1.5 ml ultrapure water, applied to a P2 column (Bio-rad, Bio-Gel P2, 1.6 cm x 50 cm), and eluted with 10 mM aqueous ammonium bicarbonate at a flow rate of 0.2 ml/min. All fractions were analysed by TLC (70:30:1 acetonitrile:water:acetic acid), which indicated that unreacted FITC and XGO-NH_2 were successfully separated from the desired product. Fractions containing XGO-FITC (detected by an on-line UV detector and TLC) and exhibiting low conductivity were pooled and concentrated *in vacuo* to afford an orange solid (yield: 34 mg, 60%). Electrospray ionization mass spectrometry (Micromass Q-TOF2) was used to confirm the identity of the modified oligosaccharides.

EXAMPLE 11

Synthesis of radioactive and non-radioactive alditol derivatives of XLLG xylogluco-oligosaccharide ([1-^{3}H]-XLLGol and [1-^{1}H]-XLLGol)

Xylogluco-oligosaccharide XLLG (8.6 µmol) was dissolved in purified water (250 µl, 18 MΩ.cm), which had been adjusted to pH 11.5 with NaOH. NaB^3H_4 (8.2 µmol, 3.76 GBq) was then added and the reaction was allowed to stand overnight at room temperature. The reaction was stopped by careful addition of glacial acetic acid until the pH of the solution was approximately 4. The solution was then allowed to stand for a period of 30 min to allow tritium gas to vent through the fume hood. Salts were removed from the product by gel filtration chromatography on Bio-Gel P-2 resin (Bio-Rad, bed volume 20 ml) with purified water (18 MΩ.cm) as the eluent. Fractions of approximately 1 ml volume were collected. The fractions were analyzed by liquid scintillation counting and thin-layer chromatography (silica gel, 7:3 acetonitrile-water eluent, ammonium molybdate/sulfuric acid stain). The fractions, which were both radioactive and contained a product with an Rf identical to XLLG were pooled. A qualitative Tollens test for reducing sugars on this product was negative, which indicated that the reduction reaction had gone to completion. The product a radioactivity of 115270 Bq/µL.

Synthesis of non-radioactive XLLGol was carried out in a manner identical to that described above except that $NaBH_4$ replaced NaB^3H_4 as the reducing agent. Evaporation of the chromatography solvent yielded the product as a white powder, which gave a proton NMR spectrum identical to that previously reported (York, W. *et al.*, Carbohydrate Research 1990, 200, 9-31). The average yield of three reactions (50 (+/-) 3%) was used to estimate the yield of the radioactive synthesis, which indicated a concentration of 1.21×10^{-9} mol/L and specific activity of 95 MBq/µL.

EXAMPLE 12

Preparation of regenerated cellulose membranes

a. Preparation of regenerated cellulose membrane from cuprammonium solution

According to the method of Okajima [Okajima, K. (1995) *Polymer Journal*, **27**(11), 1113-1122], 10g cellulose (Whatman No. 1 filter paper, UK) was dissolved in a mixture of 65g NH_4OH (20%), 12g freshly prepared $Cu(OH)_2$, 8g 10% (w/v) NaOH and 30g water to give a clear blue viscous solution at 4 °C. The solution was cast on a glass plate to give a thickness of 0.3 mm and then placed in coagulation baths maintained at 4 °C of 10% aqueous NaOH followed by 4% aqueous H_2SO_4 for 5 min each , respectively. The regenerated cellulose films obtained were washed in running water and dried on a glass plate at room temperature.

b. Preparation of regenerated cellulose membrane from aqueous NaOH/urea solution. *Bemliese*® nonwoven cloth made from cotton linters in curprammonium solution (DP= 650, Asahi Chemical Industry Co. Ltd., Japan) was used as the source of cellulose. 10 g *Bemliese*® nonwoven cloth was dissolved in 200 ml 6 wt % NaOH / 4 wt % urea aqueous solution to obtain a clear cellulose solution at 4 °C. The solution was cast on a glass plate to give a thickness of 0.5 mm, then immediately immersed into 5 wt % H_2SO_4 aqueous solution to allow coagulatation for 5 min at 4 °C. The transparent membranes obtained were washed by running water and dried in air on a glass plate at room temperature.

EXAMPLE 13

XET mediated incorporation of sulforhodamine-modified xyloglucan oligosaccharides (XGO-SR) into xyloglucan in solution

The fluorophore sulfurhodamine was chemically incorporated on the reducing end of xyloglucan oligosaccharides to produce XGO-rhodamine as described in Example 8. A mixture (4 ml) of xyloglucan (XG, 0.5 mg/ml), XGO-rhodamine (0.5 mg/ml) and XET (0.025 mg/ml) in ammonium acetate buffer (50 mM, pH 5.5) was incubated at room temperature (22°C) for 10 min. The reaction was terminated by eluting the reaction mixture through a HiTrap SP FF column (Amersham Biosciences, Sweden) to remove the XET enzyme. Approximately 25% of the added XG was modified with XGO-rhodamine, as determined by the colorimetric assay of Kooiman [Kooiman, P. (1960) *Recl. Trav. Chim. Pay-Bas*, **79**, 675-678].

EXAMPLE 14

XET mediated incorporation of fluorescein-modified xyloglucan oligosaccharides (XGO-FITC) into xyloglucan in solution

Fluorescein isothiocyanate Isomer I was chemically incorporated on the reducing end of xyloglucan oligosaccharides to produce XGO-FITC as described in Example 10. The effects of the XET enzyme concentration and the reaction time on the incorporation of XGO-FITC into xyloglucan in solution were analysed as follows.

a. Time dependence

Samples containing a mixture (200 µL total volume) of xyloglucan (XG, 1 mg/ml), XGO-FITC (0.5 mg/ml) and XET (8 units) in citrate buffer (20mM, pH 5.5) were incubated at 30

°C for 5, 10, 20, 40, 60, 120, 180, 300, and 360 min. At the appropriate time, each reaction was terminated by heating at 75 °C for 5 min. After cooling to room temperature, 400μL of ethanol was added and the mixture was centrifuged at 12000 *g* for 5 min at 4 °C to precipitate modified and unmodified XG while leaving XGO-FITC in solution. Both the precipitate and the supernatant were dried under vacuum and redissolved in 200 μL water separately. 0.019, 0.025, 0.031, 0.035, 0.038, 0.041, 0.042, 0.043, 0.044 mg of XGO-FITC were incorporated into the reducing end of xyloglucan in the 5, 10, 20, 40, 60, 120, 180, 300, and 360 min samples, respectively, as determined by the UV absorption at 495 nm of the redissolved precipitate solution using a standard line derived from XGO-FITC solutions of increasing concentration. The results are plotted in Figure 5.

b. Enzyme dependence

A mixture (200 μL total volume) of xyloglucan (XG, 1 mg/ml), XGO-FITC (0.5 mg/ml) in citrate buffer (20mM, pH 5.5) was incubated with decreasing amounts of XET (32.0, 16.0, 14.4, 12.8, 9.6, 6.4, 4.8, 3.2, 1.6, and 0.8 units) at 30 °C for 40 min. At that time, the reaction mixtures were treated exactly as described in Example 13b. Using this procedure, it was found that 0.042, 0.038, 0.038, 0.037, 0.034, 0.031, 0.027, 0.022, 0.015, and 0.009 mg XGO-FITC were incorporated to the reducing end of xyloglucan, in the samples containing 32.0, 16.0, 14.4, 12.8, 9.6, 6.4, 4.8, 3.2, 1.6, and 0.8 units of XET enzyme, respectively. The results are plotted in Figure 6.

EXAMPLE 15

Adsorption of sulforhodamine-modified xyloglucans onto cellulose materials

Cellulosic materials (0.1 g Munktell filter paper strip) were immersed in the solution containing rhodamine-modified XG (4 ml, produced according to the method in Example 10) and agitated in an end-over-end mixer overnight (ca. 15 hours) at room temperature. Binding of XG on the cellulosic fibres (11.4 mg XG / g cellulose) was analyzed by the loss of XG from solution, as determined by the colorimetric method of Kooiman. The cellulosic material was then removed from the original solution and washed repeatedly with ultrapure water in an end-over-end mixer to remove excess XGO-rhodamine. Following extensive washing, the adsorption of rhodamine-XG on cellulose was also observed as a bright pink coloration under ambient light and strong fluorescence under UV light. Control samples treated under identical conditions, but which contained only unmodified xyloglucan and XGO-SR were colorless after washing.

Example 16

XET mediated incorporation of chemically-modified xyloglucan oligosaccharides into xyloglucan pre-adsorbed on cellulose fibres

XG (0.5 mg/ml) was incubated with cellulosic fibres overnight (15 hr, gentle end-over-end mixing) to first adsorb XG onto the cellulose. The XG-cellulose was then treated with a mixture of XGO-rhodamine (0.1 mg/ml) and XET (0.025 mg/ml) in the buffer of 50 mM ammonium acetate, pH 5.5. After mixing in an end-over-end mixer at room temperature for 4 hours, the sample was washed extensively with ultrapure water. The covalent incorporation of the fluorescent oligosaccharides was evidenced by a strong pink colour on the cellulose fibres, which also showed strong fluorescence under UV light.

EXAMPLE 17

Adsorption of fluorescein-modified xyloglucan (XG-FITC) onto cellulosic paper

a. A mixture (4 ml total volume) of xyloglucan (XG, 0.5 mg/ml), XGO-FITC (0.5 mg/ml) and XET (0.025 mg/ml) in ammonium acetate buffer (25 mM, pH 5.5) was incubated at room temperature (22°C) for 10 min. The reaction was terminated by eluting the reaction mixture through a HiTrap SP FF Column (Amersham Biosciences, Sweden) to remove the XET enzyme. Cellulosic materials (0.1g Whatman No. 1 filter paper strip) were subsequently immersed in the solution and agitated in an end-over-end mixer for 15 hours at room temperature. Binding of XG on the cellulosic fibres (12.6 mg XG/g cellulose) was analyzed by the loss of XG from solution, as determined by the colorimetric method of Kooiman. The cellulosic material was then removed from the original solution and washed repeatedly with ultrapure water in and end-over-end mixer to remove excess XGO-FITC. The adsorption of XG-FITC on cellulose was observed as a bright yellow coloration under ambient light and strong fluorescence under UV light. Control samples to which no XET enzyme had been added to the XG/XGO-FITC solution were colorless and not fluorescent.

b. A mixture (200 µL) of xyloglucan (XG, 1 mg/ml), XGO-FITC (0.5 mg/ml) and XET (8 units) in citrate buffer (20mM, pH 5.5) was incubated at 30 °C for 60 min. The reaction was terminated by heating at 75 °C for 5 min. 100 µL of this solution was diluted to 500 µL in ultrapure water, and cellulosic material (Whatman No. 1 filter paper disc, diameter 1.5 cm, 15.4 mg) was immersed in the solution followed by agitation in an end-over-end mixer for 15 hours at room temperature. The filter paper was then removed and washed with ultrapure water (2 x 1ml). The amount of the XGO-FITC which was incorporated into XG and subsequently bound to the filter paper (0.0232 mg) was analyzed by the loss of XGO-

FITC from solution (including the wash solutions), as determined by UV adsorption at 495 nm in 0.1M sodium bicarbonate versus a standard line of XGO-FITC. To directly quantitate the amount of FITC modified xyloglucan (XG-FITC) on the paper surface, the paper was imaged both with a CCD camera during fluorescent excitation (Fujifilm imager) and a desktop scanner. It was found that when scanned in full-color RGB mode, the intensity of the blue channel showed a linear correlation with the amount of XG-FITC adsorbed on the paper. Furthermore, bound XG-FITC could be extracted from the paper with aqueous 2M NaOH and subsequently quantitated by UV adsorption at 495nm in 0.1M sodium bicarbonate. Treatment of XG-FITC with 2M NaOH was shown to have no effect on the UV absorbance or fluorescence emission and excitation spectra of the compound. Confocal microscopy images showed that the fluorophore was specifically localized on the fibre surfaces and demonstrates that the signal is clearly detectable in spite of the porosity of the material.

A confocal fluorescence microscopy image of XG-FITC-treated paper is shown in Figure 7. The light areas indicate high relative fluorescence intensity and darker areas indicate a lower relative fluorescence intensity.

EXAMPLE 18

Adsorption of sulforhodamine-modified (XG-SR) and fluorescein-modified xyloglucan (XG-FITC) onto regenerated cellulose membranes

a. Cellulose regenerated membranes (0.05g) were immersed into a solution containing sulforhodamine-modified XG (XG-SR, 4 ml, produced according to the method in Example 13) and agitated in an end-over-end mixer for 15 hours at room temperature. Binding of the XG-SR to regenerated cellulose membrane was determined by the loss of XG-SR from solution to be 0.3 mg/g, using the colorimetric method of Kooiman. The cellulosic material was then removed from the original solution and washed repeatedly with ultrapure water in and end-over-end mixer to remove the excess XGO-rhodamine. The adsorption of XG-rhodamine on cellulose was observed as a bright pink coloration under ambient light and strong fluorescence under UV light. Confocal fluorescence microscopy indicated that XG-SR was localised to the membrane surfaces.

b. A mixture (200 µL) of xyloglucan (XG, 1 mg/ml), XGO-FITC (0.5 mg/ml) and XET (2 µg) in citrate buffer (20mM, pH 5.5) was incubated at 30 °C for 40 min. The reaction was terminated by heating at 75 °C for 5 min. Regenerated cellulose membranes (0.05g) were immersed in the solution. The amount of the XGO-FITC, which incorporated to XG and

subsequently bound to the membrane was analyzed by the loss of XGO-FITC from solutions (including wash solutions), as determined by UV adsorption at 495 nm in 0.1M sodium bicarbonate against standard line of XGO-FITC. Confocal fluorescence microscopy indicated that XG-FITC was bound exclusively to the membrane surfaces.

EXAMPLE 19

Preparation of amino-modified xyloglucan ($XG\text{-}NH_2$)

A typically reaction consisting of 10 mg of *Tamarindus indica* xyloglucan, 3.75 mg of amino-modified xyloglucan oligosaccharides (mixture of $XXXG\text{-}NH_2$, $XLXG\text{-}NH_2$, $XXLG\text{-}NH_2$, $XLLG\text{-}NH_2$, prepared according to Example 8) and 182 units XET (49 µg protein, Bradford assay) were incubated in 20 mM citrate buffer pH 5.5 for 30 minutes at 30 °C. The enzyme was deactivated by heating to 75°C for ten minutes. The colorimetric assay of Sulová et al. (1995) *Anal. Biochem.* **229**, 80-85, typically showed a change of 0.4 adsorbance units at 620 nm after incubation, comparable to that observed when XGO-FITC was used as a substrate under similar conditions.

EXAMPLE 20

Adsorption of amino-modified xyloglucan ($XG\text{-}NH_2$) onto cellulosic paper

Amino-modified xyloglucan ($XG\text{-}NH_2$, prepared as described in Example 19) was diluted 1:1 with water and incubated with a sheet of filter paper (Whatman No. 1, 1.5 cm diameter, 15 mg) in a glass vial overnight at room temperature with orbital shaking. The paper was washed extensively with ultrapure water. Typically 70 to 80% of the amino-modified xyloglucan was adsorbed to the paper, as determined by the colorimetric method of Kooiman. The content of amino groups on the paper was quantified with ninhydrin as described by Sarin et al. (1981) *Anal. Biochem.*, **117**, 147-157, which gave typically 70-80 nmol of detected amino groups per sheet of paper.

EXAMPLE 21

Reaction of amino-modified xyloglucan ($XG\text{-}NH_2$) adsorbed on the surface of cellulosic paper with fluorescein isothiocyanate

Cellulosic paper (Whatman No. 1, 1.5 cm diameter, 15 mg) which had been prepared as described in Example 20 was incubated with fluorescein isothiocyanate, isomer I, (0.6mg)

in 500 µL 0.1 M $NaHCO_3$ overnight at room temperature in glass vials with orbital shaking. The paper was washed extensively with 0.1 M $NaHCO_3$ and ultrapure water. Paper treated in this manner appeared bright yellow under ambient light and exhibited strong fluorescence. The degree of modification was quantitated as outlined in Example 17b. Control samples of paper treated in the same manner but to which no XG-NH_2 had been added were colorless and showed no fluorescence.

A photo of the result of incorporation of fluorescein into paper treated with XG-NH_2 and reacted with FITC is shown in Figure 8. The left filter disc, darker was reacted with FITC whereas the right filter disc was treated identically to the left, except that no XG-NH_2 was bound prior to reaction with FITC. From Figure 8 it is clear that the FITC is bound to the filter disc.

EXAMPLE 22

Reaction of amino-modified xyloglucan (XG-NH_2) adsorbed on the surface of cellulosic paper with acetic anhydride

Cellulosic paper (Whatman No. 1, 1.5 cm diameter, 15 mg) which had been prepared as described in Example 20 was incubated with 3.75 mM acetic anhydride and 11 mM triethylamine in 2 ml anhydrous methanol overnight at room temperature in glass vials with orbital shaking. The paper was then washed with methanol followed by an excess of water. The amount of amino groups detected by the quantitative ninhydrin assay [Sarin et al. (1981) *Anal. Biochem.*, **117**, 147-157] decreased by 84% compared to a control sample. Acetylated paper produced in this manner was also reacted with fluorescein isothiocyanate by the manner outlined in Example 20 and quantitated according to Example 17b indicated that 100% of the amino groups had reacted versus an unmodified amino-paper control.

EXAMPLE 23

Reaction of amino-modified xyloglucan (XG-NH_2) adsorbed on the surface of cellulosic paper with phenylisocyanate

Cellulosic paper (Whatman No. 1, 1.5 cm diameter, 15 mg) which had been prepared as described in Example 20 was incubated with a 1 M solution of phenylisocyanate in methanol (2 ml) overnight at room temperature in glass vials with orbital shaking. The paper was then washed with methanol (3 x 5 ml, in vials) followed by an excess (1 L, on a

glass frit) of water. The amount of amino groups detected by the quantitative ninhydrin assay [Sarin et al. (1981) *Anal. Biochem.*, **117**, 147-157] decreased by 70% compared to a control sample. Paper produced in this manner was also reacted with fluorescein isothiocyanate by the manner outlined in Example 21 and quantitated according to Example 17b indicated that 64% of the amino groups had reacted versus an unmodified amino-paper control.

EXAMPLE 24

Reaction of amino-modified xyloglucan (XG-NH_2) adsorbed on the surface of cellulosic paper with alkenyl succinic anhydride (ASA) in dimethylsulfoxide (DMSO)

Cellulosic paper (Whatman No. 1, 1.5 cm diameter, 15 mg) which had been prepared as described in Example 20 was incubated with a 4 mM solution of ASA in DMSO (2 ml) overnight at room temperature in glass vials with orbital shaking. The paper was washed two times with 10 ml 2-propanol, two times with 10 ml methanol, and two times with 10 ml of water in the vial and finally washed with 1 L of purified water on a glass fritt. The amount of detected amino groups decreased by 63% compared to a untreated control sample. Paper produced in this manner was also reacted with fluorescein isothiocyanate by the manner outlined in Example 21 and quantitated according to Example 17b indicated that 63% of the amino groups had reacted versus an unmodified amino-paper control.

EXAMPLE 25

Reaction of amino-modified xyloglucan (XG-NH2) adsorbed with on the surface of cellulosic paper with succinic acid anhydride in methanol (MeOH)

Cellulosic paper (Whatman No. 1, 1.5 cm diameter, 15 mg) which had been prepared as described in Example 20 was incubated with a 1.5 M solution of succinic acid anhydrite in anhydrous MeOH (2 ml) overnight at room temperature in glass vials with orbital shaking. The paper was washed two times with 10 ml methanol, two times with 10 ml of purified water in the vial and finally washed with 1l of purified water on a glass frit. The amount of detected amino groups decreased by 48% compared to a untreated control sample. Paper produced in this manner was also reacted with fluorescein isothiocyanate by the manner outlined in Example 21 and quantitated according to Example 17b indicated that 38% of the amino groups had reacted versus an unmodified amino-paper control.

EXAMPLE 26

Reaction of amino-modified xyloglucan (XG-NH_2) adsorbed on the surface of cellulosic paper with *N*-cinnamoyl imidazole

Cellulosic paper (Whatman No. 1, 1.5 cm diameter, 15 mg), which had been prepared as described in Example 20, was incubated with 1 M *N*-cinnamoyl imidazole in dimethylsulfoxide (2 ml) overnight at room temperature in glass vials with orbital shaking. The paper was then washed with 2-propanol (2 x 5ml), methanol (2 x 5ml), and an excess of water (1 L). The amount of amino groups detected by the quantitative ninhydrin assay [Sarin et al. (1981) *Anal. Biochem.*, **117**, 147-157] decreased by 65% compared to a control sample. Paper produced in this manner was also reacted with fluorescein isothiocyanate by the manner outlined in Example 21 and quantitated according to Example 17b indicated that 84% of the amino groups had reacted versus an unmodified amino-paper control.

EXAMPLE 27

Reaction of amino-modified xyloglucan (XG-NH_2) adsorbed on the surface of cellulosic paper with bromo isobutyric acid

Cellulosic paper (Whatman No. 1, 1.5 cm diameter, 15 mg) which had been prepared as described in Example 20 was incubated with a solution containing 1 M bromo isobutyric acid and 1 M 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide in MeOH (2 ml) overnight at room temperature in glass vials with orbital shaking. The paper was washed with methanol (2 x 10 ml) and ultrapure water (2 x 10 ml) in the vial and finally washed with 1 L of ultrapure water on a glass fritt. The amount of detected amino groups by 50% compared to a untreated control sample. Paper produced in this manner was also reacted with fluorescein isothiocyanate by the manner outlined in Example 21 and quantitated according to Example 17b indicated that 79% of the amino groups had reacted versus an unmodified amino-paper control.

EXAMPLE 28

Reaction of amino-modified xyloglucan (XG-NH_2) adsorbed on the surface of cellulosic paper with biotin 3-sulfo-*N*-hydroxycuccinimide ester (succinimidyl biotin)

Cellulosic paper (Whatman No. 1, 1.5 cm diameter, 15 mg), which had been prepared as described in Example 20, was incubated with a solution containing 180 µM solution of succinimidyl biotin in 10 mM $NaHCO_3$ (2 ml) overnight at room temperature in glass vials with orbital shaking. The paper was washed four times with 10 ml of purified water in the vial and finally washed with 1 L of purified water on a glass fritt. The amount of detected amino groups decreased by 71% compared to a untreated control sample. Paper produced in this manner was also reacted with fluorescein isothiocyanate by the manner outlined in Example 21 and quantitated according to Example 17b indicated that 57% of the amino groups had reacted versus an unmodified amino-paper control.

The biotinylated paper and a control sample were incubated over night with 500 µL of a 0.1% BSA solution with end over end shaking to block non-specific protein binding to the paper. The paper was washed two times with 1 ml of water and incubated with 10 µg streptavidin-alkaline phosphatase conjugate in 100 mM Tris, pH 9.5, for 15 min at room temperature. The paper was washed 4 times with 1 ml of the same Tris buffer. The paper was then incubated with 18 µg 5-bromo-4-chloro-3-indolylphosphate/nitro blue (BCIP/NBT) in 320 µL Tris buffer pH 9.5 for 5 min. The paper was subsequently washed with two times with 20 µL purified water and dried. The paper was subjected to image analysis after scanning on a desktop image scanner. The visibly blue paper was 40% more colored than the control, non-biotinylated amino paper.

EXAMPLE 29

Reaction of amino-modified xyloglucan ($XG-NH_2$) adsorbed on the surface of cellulosic paper with γ-thiobutyrolactone

Cellulosic paper (Whatman No. 1, 1.5 cm diameter, 15 mg) which had been prepared as described in Example 20 was reacted with γ-thiobutyrolactone (87 µl, 1mol) in a mixture of ethanol (98%, 500µl) and sodium bicarbonate aqueous solution (0.1 M, 500µl) overnight at room temperature in glass vials with orbital shaking. The paper was washed 2 times with 5 ml 0.1 M sodium bicarbonate aqueous solution and finally with approximately 1 L pure water. The amount of detected amino groups decreased by 52% compared to an untreated control sample.

Figure 9 shows the relative amounts of amino groups present on the surface of $XG-NH_2$-modified cellulosic paper following treatment with various amino-reactive reagents. Light grey bars relates to determination by quantitative ninhydrin assay whereas dark grey bars relates to quantiation by reaction with FITC followed by quantitative image analysis. Figure 10 shows the reactivity of paper with and without modification by $XG-NH_2$ toward FITC. "Blank" is commercial filter paper treated with FITC; "XGN" is commercial filter paper treated with $XG-NH_2$ followed by FITC; "1" is commercial filter paper treated with $XG-NH_2$ followed by acetic anhydride, then FITC.

EXAMPLE 30

Reaction of Sulfo-rhodamine methanethiosulfonate with the thiol groups introduced onto the surface of cellulosic paper

Half of the dry cellulosic paper (7.7 mg), which was produced as described in Example 29, was treated with 2 ml of 10 mM dithiothreitol (DTT) in 0.1 M $NaHCO_3$ aqueous solution under argon in a glass vial for 2 hours with occasional shaking. After the paper was washed 3 times with 2 ml degassed ultrapure water under argon, 1 ml of 1mM sulfo-rhodamine methanethiosulfonate in a solution of DMSO/H_2O (1:9) was added and allowed to react for 2 hours with occasional shaking. The paper was washed with DMSO to remove the unreacted sulfo-rhodamine methanethiosulfonate, washed with ultrapure water, and dried.

The paper exhibited a bright pink coloration under ambient light and a strong fluorescence under UV light, while the blank paper treated in the same way was colorless.
Half of the pink paper (ca. 3 mg) was treated with 500 µL of 10 mM dithiothreitol (DTT) in 0.1 M $NaHCO_3$ aqueous solution again to release the disulfide-bonded sulfo-rhodamine methanethiosulfonate from the paper surface. The supernatant solution was quantitated by UV adsorption at 565 nm. The paper was washed 3 times with 1 ml degassed water under argon and reacted again with 1 ml of 1mM sulfo-rhodamine methanethiosulfonate in a solution of DMSO/H_2O (1:9) mixture for 2 hours with occasional shaking. After washing with DMSO and water, the paper again exhibited a bright pink coloration under ambient light and a strong fluorescence under UV light, while the blank paper treated in an identical manner was colorless.

Figure 11 shows the result of reaction of thiolated paper with sulforhodamine methanethiosulfonate. Samples on the left in each row represent XG-NH_2-treated paper, while those on the right represent XG-NH_2-treated paper, which was subsequently reaction with thiobutyrolactone to introduce a thiol (-SH) group. Top row: samples after treatment with sulforhodamine methanethiosulfonate. Middle row: portions of samples from the top row after washing with DTT solution. Bottom row: DTT washed samples, which were reacted again with sulforhodamine methanethiosulfonate.

EXAMPLE 31

Atom transfer radical polymerization using initiator coupler with XG on the cellulosic surface

Atom transfer radical polymerization from cellulose paper surfaces at ambient temperatures has been described [Carlmark and Malmström, 2002, *J. Am. Chem. Soc.* **124**: 900-901], however at high loading amounts of initiator, paper integrity is severely compromised. We carried out the same polymerization reaction from initiator immobilized on the cellulose surface via xyloglucan as described in Example 27, a procedure which gave high levels of initiator on the paper surface with no degradation of paper structure. The graft copolymer so produced showed dramatically increased fibre-polymer bonding compared to control samples where initiator was not coupled onto the fibre surface.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention as set forth in the claims appended hereto.

CLAIMS

1. A method of modifying a polymeric carbohydrate material (PCM), the method comprising a step of binding a chemical group having a desired functionality to said carbohydrate material by means of a carbohydrate linker molecule (CLM) comprising the chemical group, said linker molecule being capable of binding to the PCM.

2. A method according to claim 1 comprising the steps of

(i) providing a carbohydrate polymer fragment (CPF) comprising a chemical group having a desired functionality

(ii) bringing said CPF carrying the chemical group into contact with a soluble polymeric carbohydrate (SCP) under conditions leading to the formation of a complex consisting of said CPF comprising the chemical group, and the SCP, said CPF and SCP together forming the CLM, and

(iii) contacting said complex with the PCM to be modified under conditions where the complex binds to the PCM to obtain the modified polymeric carbohydrate material.

3. The method of claim 2 wherein the polymeric carbohydrate material (PCM) to be modified is a water-insoluble polysaccharide.

4. The method of claim 1 or 2 wherein the PCM to be modified is derived from a plant selected from the group consisting of a monocotyledonous plant and a dicotyledonous plant.

5. The method of claim 4 wherein the monocotyledonous plant is a plant of the family *Gramineae*.

6. The method of claim 4 wherein the dicotyledonous plant is selected from the group consisting of angiospermous plants (hardwoods), coniferous plants (softwoods) and plants belonging to the *Gossypium* family.

7. The method of any of claims 1-6 wherein the PCM in the form of cellulosic plant fibres.

8. The method of any of claims 1-6 wherein the PCM is in the form of cellulosic microfibrils derived from cellulosic plant fibres or from a bacterium.

9. The method of any of claims 1-8 wherein the SCP forms a part of the PCM to be modified.

10. The method of any of claims 1-8 wherein the SCP is not associated with the PCM to be modified.

11. The method of claim 9 or 10 wherein the SCP comprising a component selected from the group consisting of a hemicellulose, a xyloglucan, a pectin and a starch.

12. The method of any of claims 1-10 wherein the carbohydrate polymer fragment (CPF) is a fragment derived from a SCP as defined in claim 11 and containing from 2 to about 5000 polymer backbone monosaccharide units.

13. The method of claim 12 wherein the CPF is derived from xyloglucan.

14. The method of claim 13 wherein the CPF contains from 3 to about 100 including from 4 to 10 polymer backbone monosaccharide units.

15. The method of any of claims 1-14 wherein in step (ii) the CPF comprising the chemical group is brought into contact with the soluble polymeric carbohydrate (SCP) in the presence of an enzyme that is capable of promoting the formation of the complex consisting of said CPF comprising the chemical group, and at least a part of the SCP.

16. The method of claim 15 wherein the enzyme is capable of transferring native or chemically modified mono- or oligosaccharides onto an oligo- and/or polysaccharide

17. The method of claim 15 wherein the enzyme is an enzyme having transglycosylation activity.

18. The method of claim 16 or 17, wherein the enzyme, when assayed with a suitable glycosyl donor substrate in the presence and absence of a mono-, oligo-, or polysaccharide acceptor substrate under appropriate conditions to maintain enzyme activity, exhibits a rate of incorporation of the acceptor substrate into the donor substrate which is at least 10% of the hydrolytic rate, such as at least 15%, 20%, 25%, 30%, 40%, 50% or 75%, such as at least 100%.

19. The method of claim 18, wherein the glycosyl donor substrate is a xyloglucan and the acceptor substrate is a xyloglucan-oligosaccharide.

20. The method of any of the claims 18-19, wherein the assay consists of the following steps

i) incubating 0.1 mg xyloglucan, 0.1 mg xyloglucan oligosaccharides (mixture of XXXG, XLXG, XXLG, and XLLG; 15:7:32:46 weight ratio) in 200 µL 40 mM citrate buffer pH 5.5 for 30 minutes at 30 °C.

ii) stopping the reaction with 100 µL 1M HCl,

iii) the ionic strength was adjusted by adding 800 µL 20% Na_2SO_4 and 200 µL of an I_2 (0.5 % I_2, 1% KI, w/w) solution

iv) measuring the absorbance was measured at 620 nm

v) performing the steps i)-iv) without adding the xyloglucan oligosaccharides (XGO) of step i)

vi) calculating the absorbance increase in percent between from the incubation with XGO to the incubation without XGO.

21. The method of claims any of claims 16-20 wherein the enzyme is selected from the group consisting of a transglycosylase, a glycosyl hydrolase, a glycosyl transferase.

22. The method of any of claims 16-21 wherein the enzyme is a wild type enzyme or a functionally and/or structurally modified enzyme derived from such wild type enzyme.

23. The method of any of claims 16-22 wherein the enzyme is a xyloglucan endotransglycosylase (XET, EC 2.4.1.207).

24. The method of any of claims 16-23 wherein the enzyme having transglycosylation activity is derived from a plant including a plant belonging to the family *Brassica* and a plant of a *Populus* species.

25. The method of any of claims 16-24 wherein the enzyme having transglycosylation activity is produced recombinantly.

26. The method of any of claims 1-21 wherein the chemical group having a desired functionality is selected from the group consisting of an ionic group, a hydrophobic group, an uncharged hydrophilic group, a reactive group, a nucleophile, a polymerisable monomer, a chromophoric group, a fluorophoric group, biotin, a radioactive isotope, a free-radical precursor, a stable free radical moiety, a protein and a protein binding agent.

27. The method of claim 26 wherein the chemical group is an amine group.

28. The method of any of claims 1-27 wherein the obtained modified polymeric carbohydrate material (PMC) has, relative to the non-modified material, altered surface properties.

29. The method of any of claims 1-27 wherein the obtained modified polymeric carbohydrate material (PMC) has, relative to the non-modified material, altered strength properties.

30. The method of any of claims 1-27 wherein the obtained modified polymeric carbohydrate material (PMC) has, relative to the non-modified material, altered water repellence properties.

31. The method according to claim 1 wherein the linker group consists of a SCP as defined in any of claims 9-11.

32. A modified polymeric carbohydrate material (mPCM) obtainable by the method of any of claims 1-31, the material having bound thereto chemical groups having a desired functionality, said binding is mediated by a carbohydrate linker molecule that is capable of binding to the PCM.

33. The material of claim 32 which is in the form of cellulosic plant fibres or cellulosic microfibrils derived from cellulosic plant fibres or from a bacterium.

34. The material of claim 32 or 33 where the chemical groups are reactive groups capable of binding other functional groups.

35. The material of any of claims 32-34 having bound thereto two or more different types of chemical groups.

36. A composite material comprising the material of any of claims 32-35.

37. Use of the material of any of claims 32-35 or the composite material of claim 36 in manufacturing of paper and cardboard product.

38. Use of the material of any of claims 32-35 or the composite material of claim 36 as an auxiliary agent in a diagnostic or chemical assay or process.

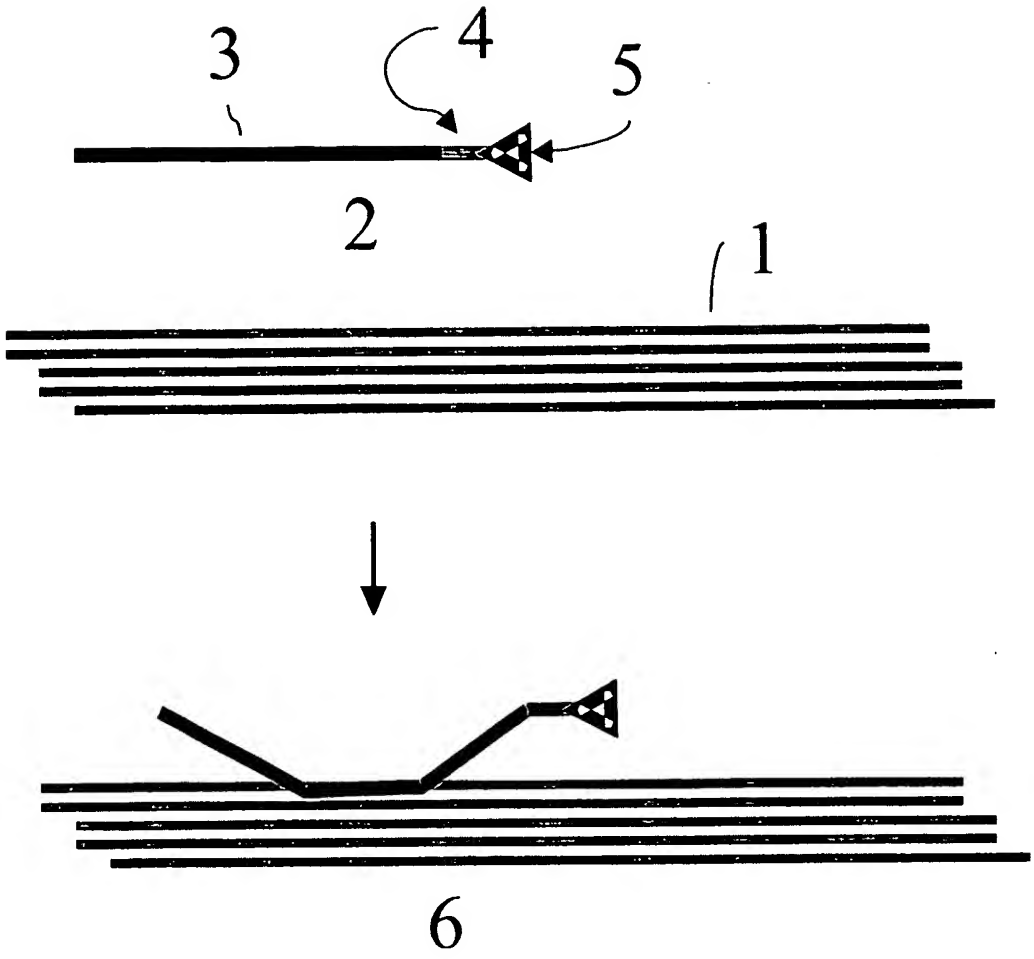
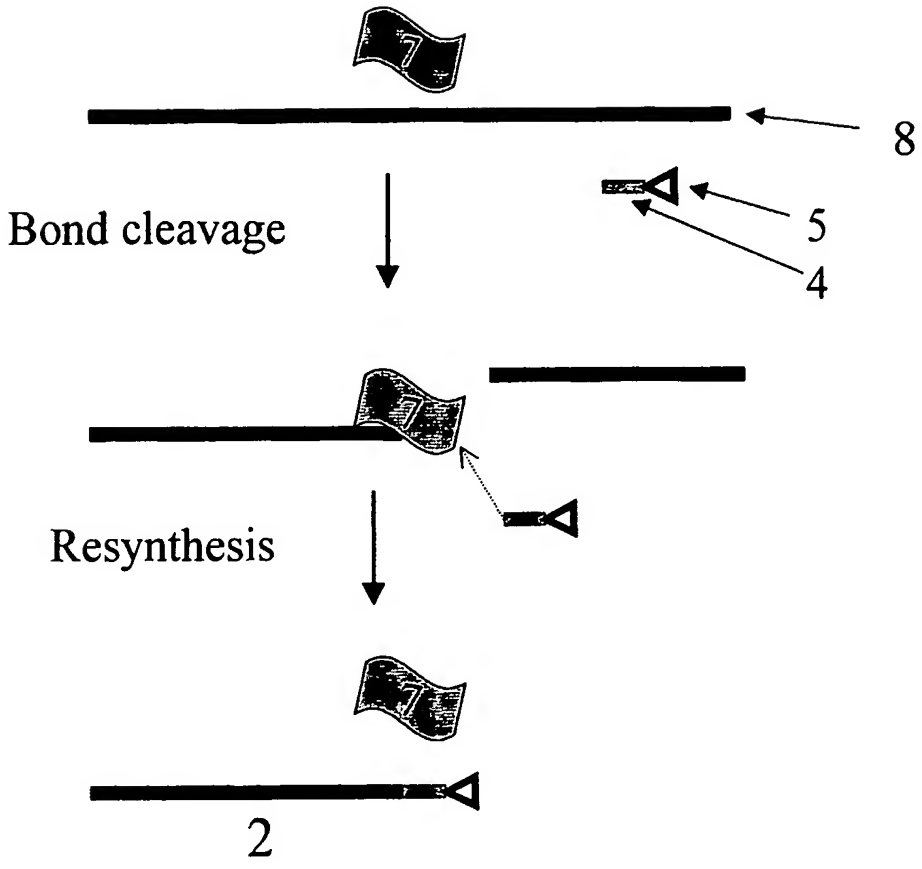


Fig. 1

PCT/IB02/04567



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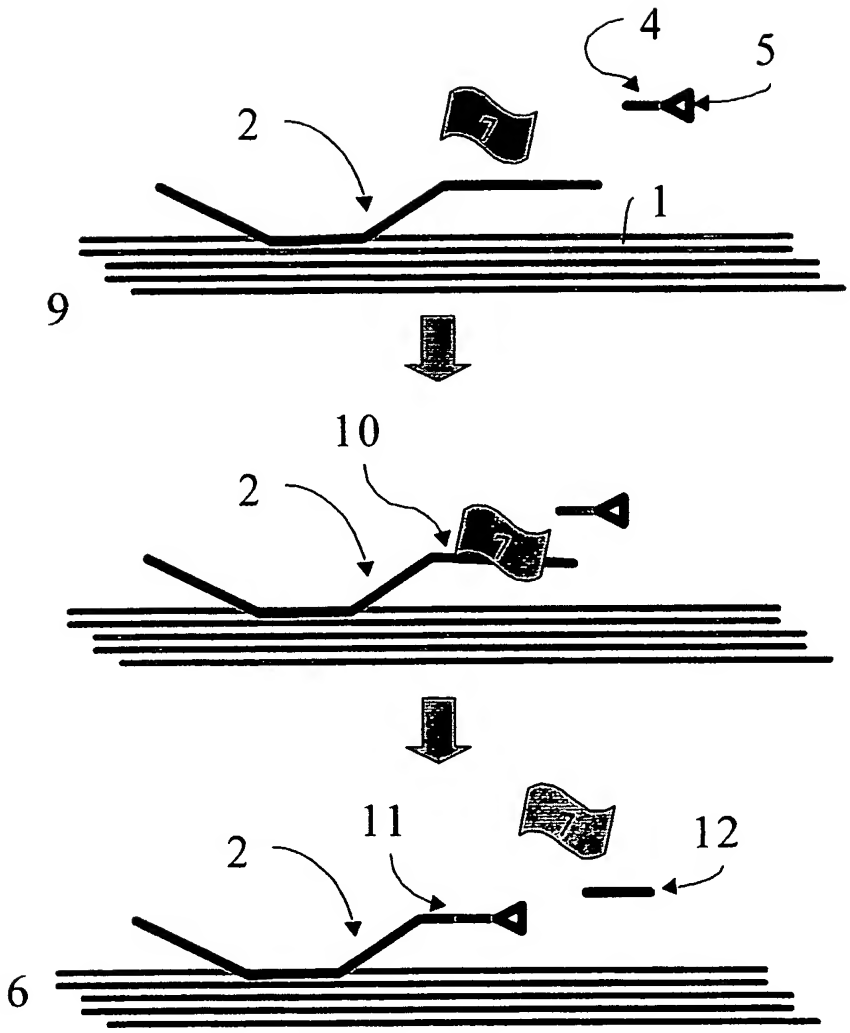

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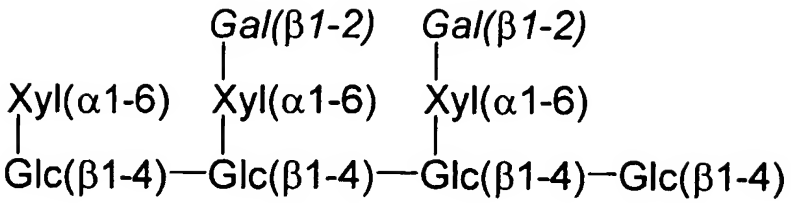

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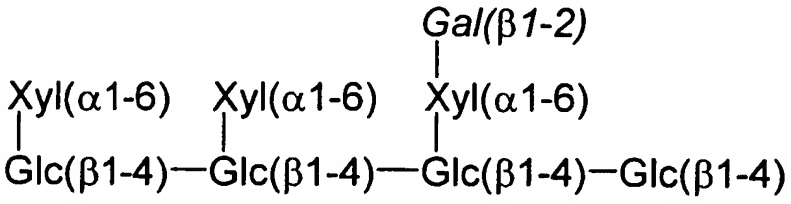

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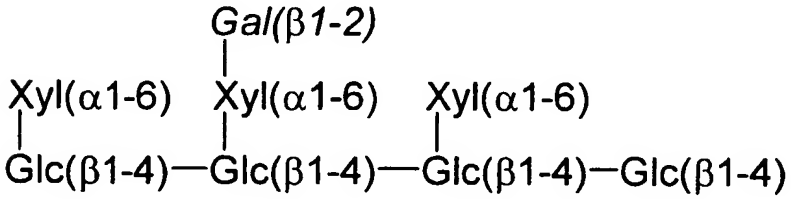

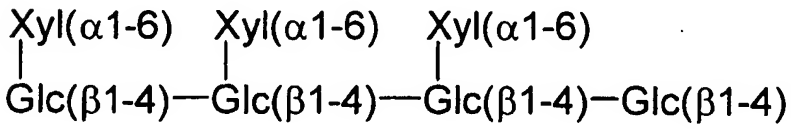

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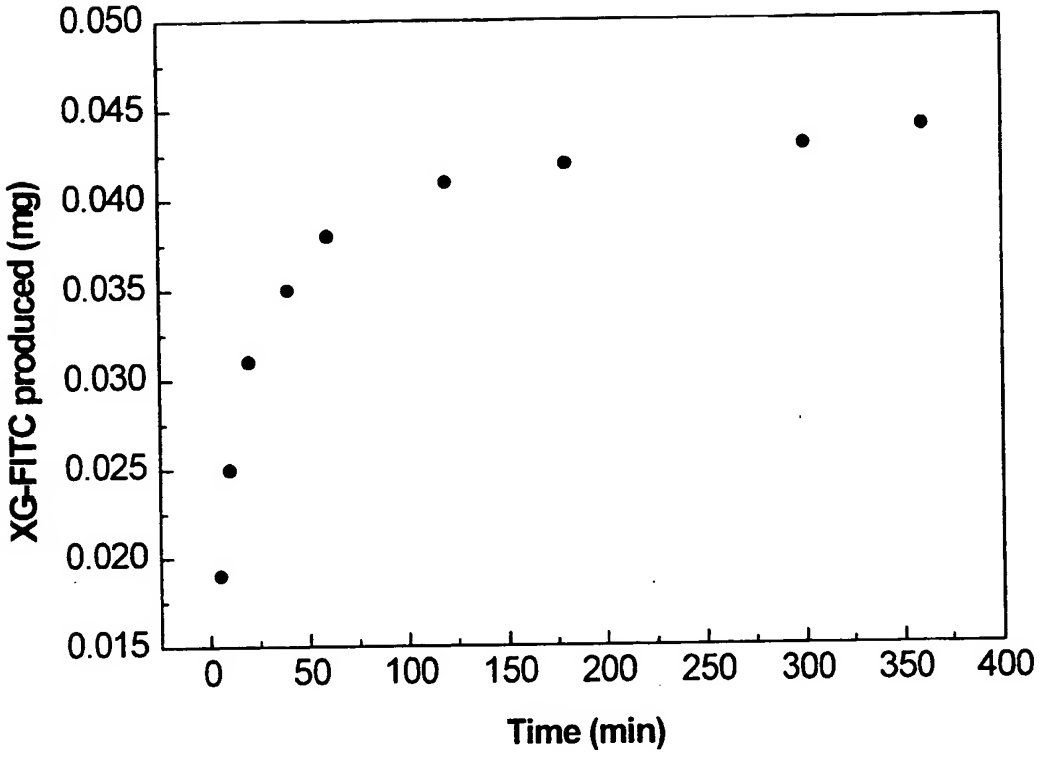
Fig. 4

PCT/IB02/04567



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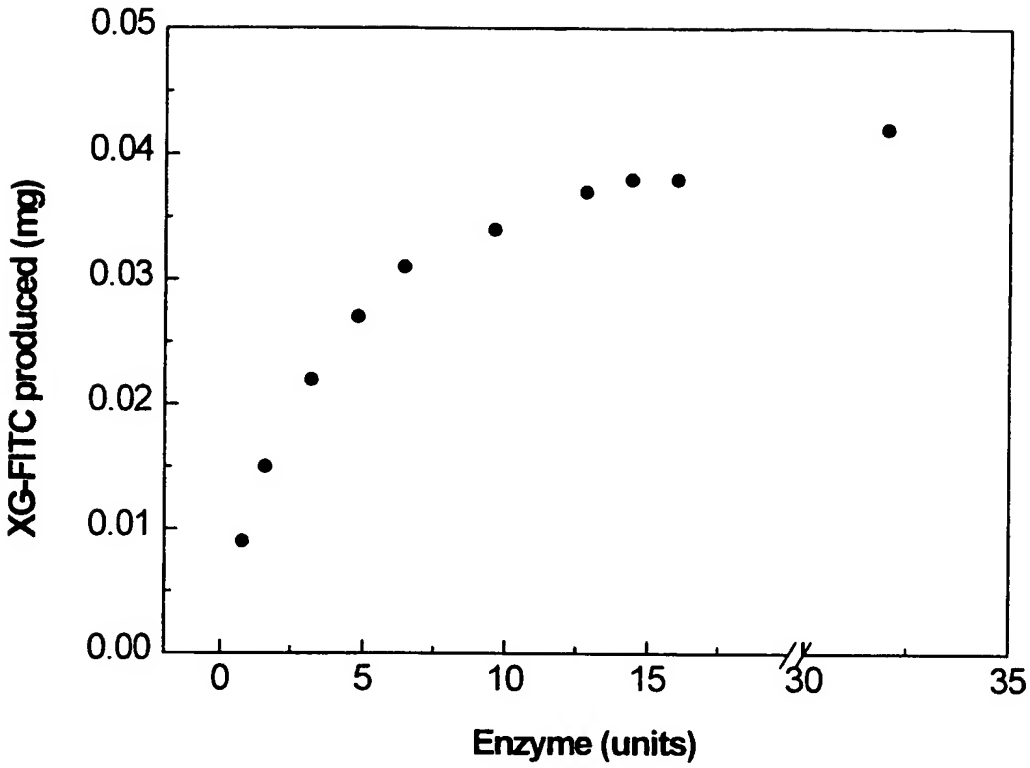

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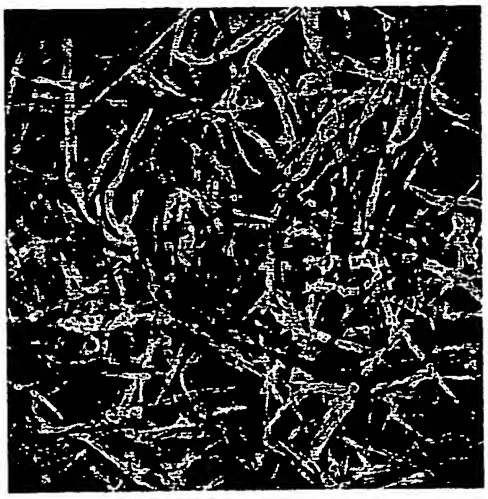

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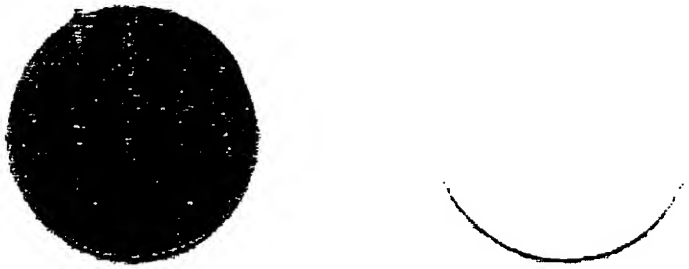

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PCT/IB02/04567

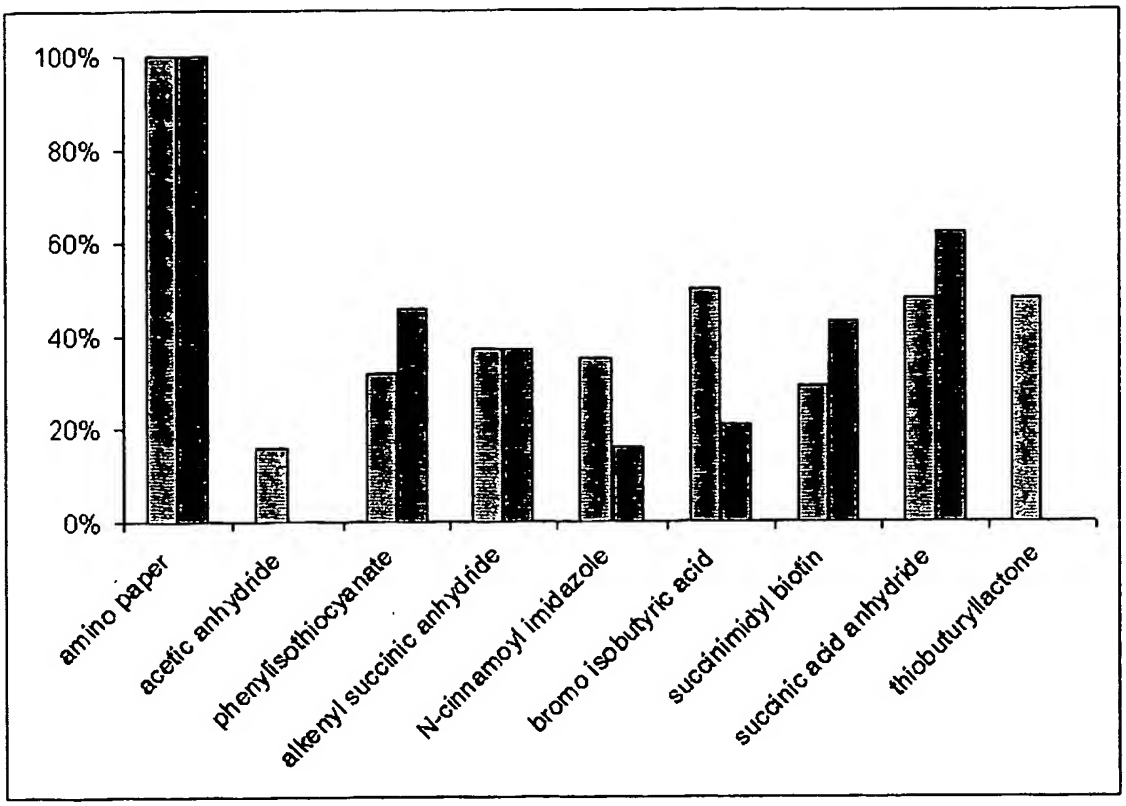

Fig. 9

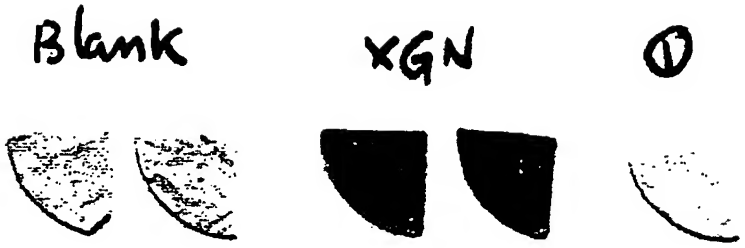

Fig. 10

PCT/IB02/04567

Fig. 11

SEQUENCE LISTING

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 02/04567

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: D06M 16/00, D06M 101/06, D06M 15/03, C12N 9/10, C08B 37/00
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C08B, D21H, D06M, C12N, C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI DATA, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5961783 A (JAMES H. PAYTON ET AL), 5 October 1999 (05.10.99), column 7, line 37 - column 8, line 11, abstract --	1
Y	WO 0107556 A1 (THE PROCTER & GAMBLE COMPANY), 1 February 2001 (01.02.01), page 1, line 11; page 6, line 20 - page 7, line 18, page 8, line 1 - line 10 (especially lines 17-19), abstract --	1-38
Y	WO 9707203 A1 (HERCULES INCORPORATED), 27 February 1997 (27.02.97), page 4, line 18 - line 23; page 5, line 8 - line 14; page 7, line 5 - line 28, page 8, line 34 - line 35; page 10, line 1 - line 11 --	1-15,26-38

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 February 2003	25-02-2003
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Marie Karlsson/Els Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 02/04567

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Protein Expression and Purification, Volume 16, 1999, Zdena Sulová et al, "Purification of Xyloglucan Endotransglyclosylase Based on Affinity Sorption of the Active Glycosyl-Enzyme Intermediate Complex to Cellulose" page 231 - page 235	16-25
	--	
A	Plant Cell Physiol, Volume 35, No 8, 1994, Takahisa Hayashi et al, "Characterization of the adsorption of Xyloglucan to Cellulose" page 1199 - page 1205	11
	--	
A	WO 9723683 A1 (NOVO NORDISK A/S), 3 July 1997 (03.07.97), page 5, line 1 - line 10	15-25
	--	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB02/04567

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

See next page

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB02/04567

The present application fails to comply with the clarity and conciseness requirements of Article 6 PCT. Claim 1 do not clearly define the matter for which protection is sought. A novelty search covering the claimed scope is not possible for the following reason:

Present claim 1 relates to a large number of possible polymeric materials and chemical groups. It is not possible to carry out a search covering all aspects of the claim.

INTERNATIONAL SEARCH REPORT

Information on patent family members

30/12/02

International application No.
PCT/IB 02/04567

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5961783 A	05/10/99	AU 7819998 A	21/12/98
		EP 0986673 A	22/03/00
		WO 9855696 A	10/12/98
WO 0107556 A1	01/02/01	AU 6377800 A	13/02/01
		EP 1198550 A	24/04/02
		US 2002151451 A	17/10/02
WO 9707203 A1	27/02/97	AU 6750296 A	12/03/97
		AU 6824896 A	12/03/97
		BR 9610219 A	15/06/99
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